ABOUT THIS USER GUIDE

This user guide is a practical guide to solving crystal structures from powder diffraction data using DASH. It includes instructions in using the Windows Interface as well as providing help on the scientific issues relevant to structure solution. It is intended for readers who already have some crystallographic experience, but perhaps with single-crystal rather than powder techniques.

Use the navigational buttons above to move between pages of the user guide and to access the full table of contents (TOC) and index.

An extensive set of tutorials are also available for DASH. Tutorial 1 will guide you through the process of structure solution in considerable detail; subsequent tutorial examples will be more concise, but will introduce other, new aspects of the structure solution process. Tutorials can be accessed by clicking on the **Tutorials** (see page 191) link on the bottom of this page.

The DASH user guide is divided into the following sections:

1 INTRODUCTION

1.1 The DASH Program

DASH solves crystal structures from powder diffraction data. It assumes that the molecular formula of the compound being studied is known, and that the unit cell and space group can be determined by indexing the powder pattern. It constructs a trial crystal structure by placing a 3D model of the compound inside the unit cell. This 3D model will generally consist of rigid units connected by links having unknown torsion angles, i.e. an accurate description of the molecule, but with an unknown molecular conformation. The chances of choosing the correct conformation and positioning the model at the correct point in the cell with the correct orientation are very small. However, DASH checks how close a trial solution is to the correct structure by calculating diffraction data and comparing it with the measured diffraction data. DASH uses simulated annealing to adjust the trial structure until it agrees well with the measured data, thereby solving the crystal structure directly from the powder diffraction data.

1.2 Basic Steps for Structure Solution

The basic steps involved in solving structures from powder data are:

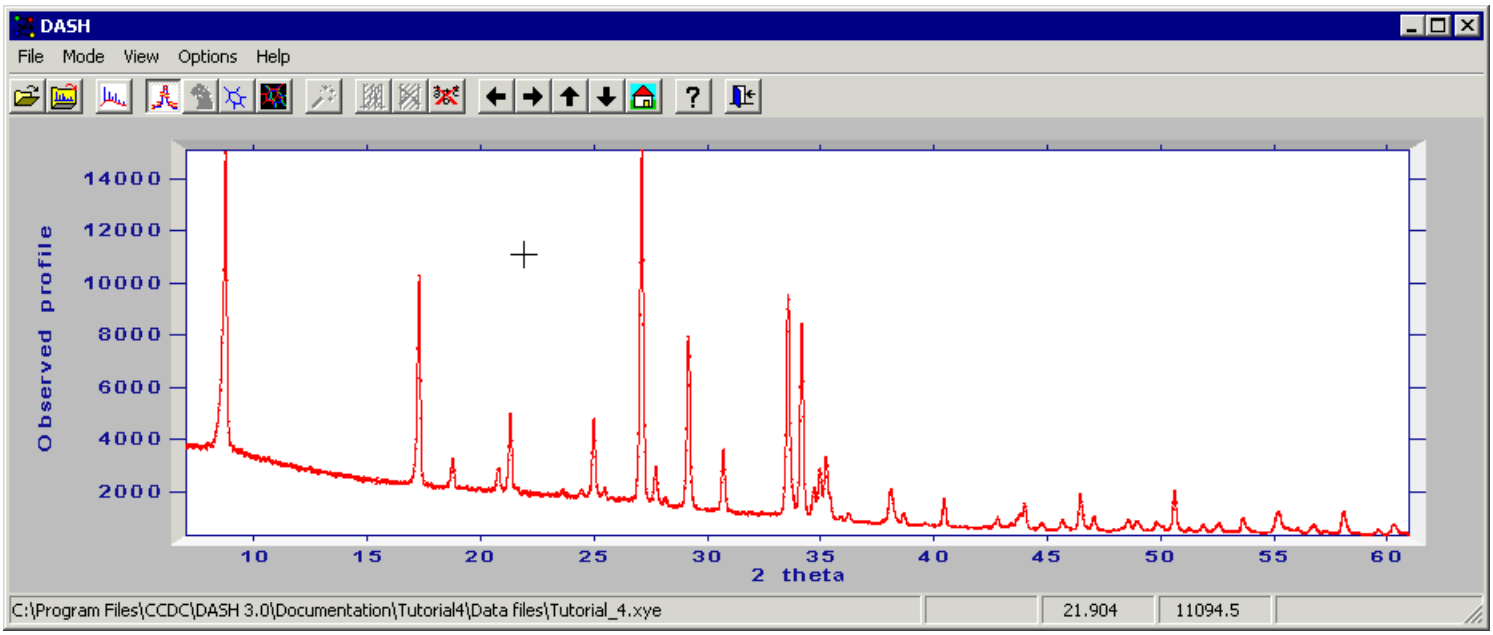
- Data collection and data treatment (see Section 3, page 31)
- Cell indexing (see Section 6, page 55) and space group determination (see Section 7, page 65)
- Extraction of reflection intensities by Pawley fitting (see Section 8, page 69).
- Building and constraining the molecules (see Section 9, page 93)
- Use of simulated annealing to solve the structure (see Section 10, page 101)

2 GENERAL FEATURES OF THE WINDOWS INTERFACE

2.1 Overview of the Windows Interface

The DASH Windows interface enables you to carry out all the necessary steps for structure solution. This section explains the layout of the main window and the various input and output files.

This is an example of the main window after reading in an X-ray diffraction pattern of laboratory data for Decafluoroquaterphenyl (Smrcok, L. et al., *Z. Kristallogr.* (2001) **216**, 63-66):



There are three ways of accessing most functions in DASH:

- Through the Wizard, use of the Wizard is highly recommended.
- From the top level menu: **File**, **Mode**, **View**, **Options** and **Help**.
- Using the Icon buttons, which provide access to functions with one mouse click.

The main window displays the experimental diffraction profile and, when applicable, the background, any selected peaks, the calculated profile, the difference profile and the cumulative χ^2, with various colour coding conventions. The *path* to the current diffraction data file is shown at the bottom left of the status bar. The coordinates of the current mouse-cursor position and the *h*, *k*, *l* values of the peak nearest to the mouse cursor are shown at the bottom right of the status bar.

2.2 Input of a Powder Diffraction File

To input a powder diffraction file, it is recommended that you use the option **View data / determine peak positions** from the main Wizard window (see Section 2.10, page 21). Alternatively, you can use

the Wizard option **Preparation for Pawley refinement,** or click on the following icon:

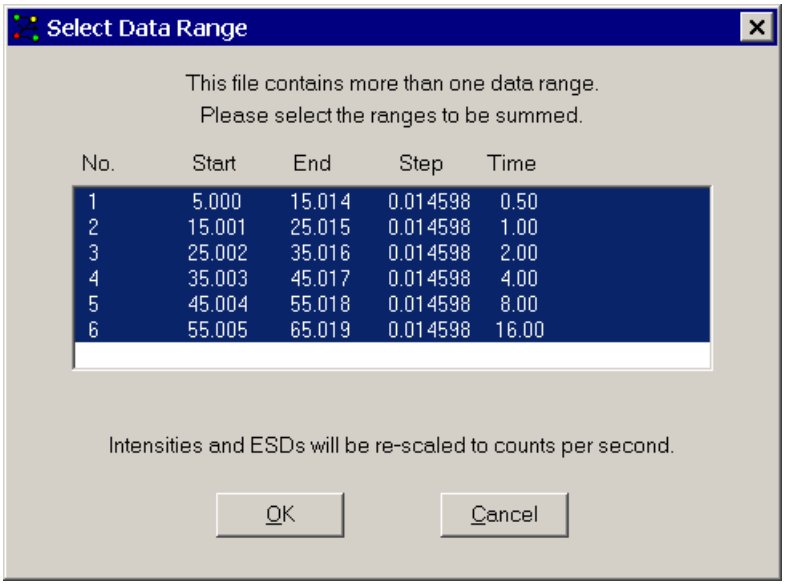
This leads to a pop-up window that shows all files in the selected directory with an extension that match those of the file types that can be read by DASH. These include:

- `.raw` - Bruker (more information about this file type is given below)
- `.raw` - STOE (more information about this file type is given below)
- `.rd` and `.sd` - Philips
- `.udf`
- `.uxd`
- `.xye`
- `.cpi` - Sietronics
- `.mdi` - Materials Data Inc.
- `.pod` - Daresbury
- `.x01` - Bede
- `.txt` - ThermoARL
- `.asc` - Rigaku

Simply:

- Click on the file icon/filename (this places the filename in the box).
- Click on **Open**.

When a Bruker `.raw` file is opened, DASH scans the file for data ranges. When only one is found, it is loaded, but when more than one is found, the data ranges to be summed can be selected through the following dialogue window:



By default all data ranges are selected and clicking **OK** reads in all data ranges.

DASH sums the data ranges as follows:

1. Per 2q value (= data point), the number of raw counts and the number of seconds that has been counted for is stored.
2. The combined list of 2q values from all data ranges is sorted in ascending order.
3. If two 2q values are closer together than the smallest 2q step used in any of the patterns being summed they are merged by summing their raw numbers of counts, summing their counting times and averaging their 2q values. This process is repeated until the original smallest step size is restored.
4. The intensities are scaled to Counts Per Second by dividing the total raw number of counts per 2q value by the total number of seconds counted for that 2q value. The esds are calculated as (total raw number of counts)$^{1/2}$ / (total number of seconds counted for).

It is possible to implement an optimised data collection strategy (see Section 3.1.12, page 34) using multiple data ranges in Bruker `.raw` files. In an optimised data collection strategy data points at higher 2q, which on average have less intensity, are measured longer than data points at lower 2q. The Variable Counting Time scheme used for the above file would have been:

2q range	counting time (seconds per step)
5.0 - 15.0	0.5
15.0 - 25.0	1.0
25.0 - 35.0	2.0
35.0 - 45.0	4.0
45.0 - 55.0	8.0
55.0 - 65.0	16.0

The third step is performed to smooth the seams between two adjacent data ranges, but it also allows reading in of data that has been collected using the following Variable Counting Time scheme, entirely equivalent to the previous one:

2q range	counting time (seconds per step)
5.0 - 65.0	0.5
15.0 - 65.0	0.5
25.0 - 65.0	1.0
35.0 - 65.0	2.0
45.0 - 65.0	4.0
55.0 - 65.0	8.0

2.3 Format of Diffraction Data

DASH recognises two formats of diffraction data within the file type with extension `.xye` (the file should be a normal ASCII text file):

- 2q, counter reading, estimated standard deviation of the count.
- 2q, counter reading.

If no standard deviation is given in the input file DASH recognises this and sets the standard deviation to be the square root of the count. The start of an example file, where the diffractometer step size is 0.004 degrees 2q, is given below:

```
5.000 81.96 10.952
5.004 71.25 10.284
5.008 72.40 10.343
5.012 76.87 10.661
5.016 63.58 9.695
```

Optionally, the wavelength can be included in the file as the very first line.

2.4 Inspecting Diffraction Data

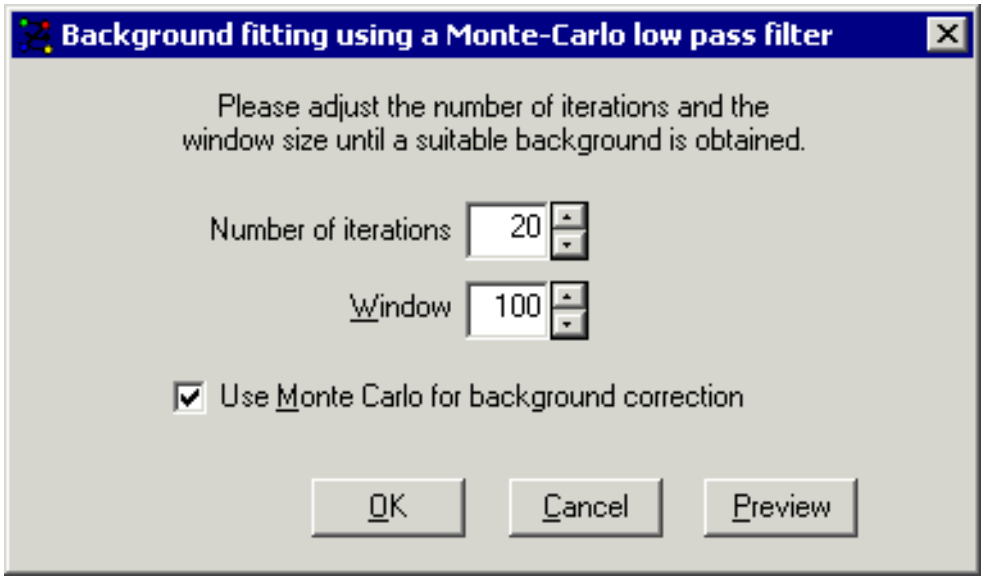
The main window shows the diffraction data plotted as observed counts versus 2q. There are numerous keyboard and mouse facilities for selecting ranges of 2q, scaling of peak height, and generally zooming into a region of the profile for closer examination (see Section 4, page 37). The next stage for consideration is removal of the background from the data (see Section 2.4.1, page 9).

2.4.1 Removing the Background from Diffraction Data

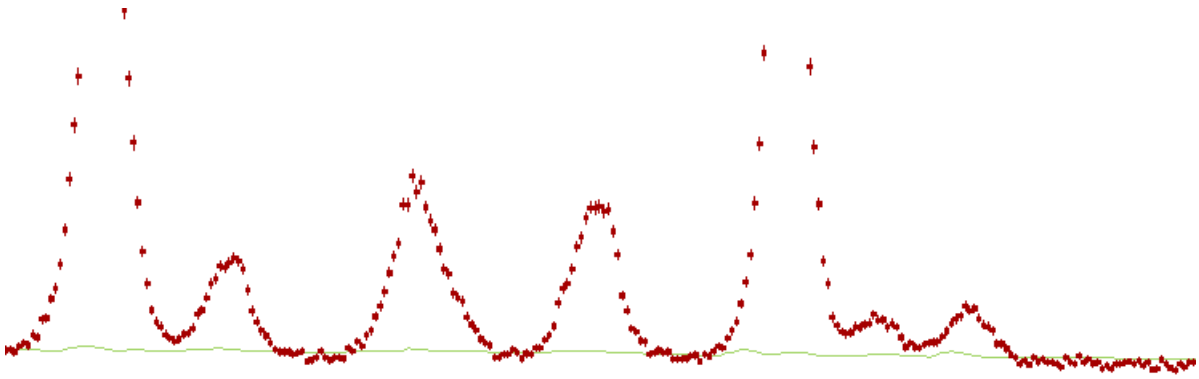
It is recommended that the background is subtracted through the Wizard (see Section 2.10, page 21). Alternatively the background can be subtracted choosing the following icon from the menu bar:

The method of background removal is a Monte Carlo low-pass filter, which has advantages of being mathematically robust. It is recommended that you let DASH take care of the background subtraction at this stage. If you choose to leave the background fitting to later, it will be included in the Pawley fitting process, using a shifted Chebyshev polynomial (see Section 8.5.1, page 79).

Note: If your data set is of laboratory origin, or there is noticeable non-uniform background then it is best to remove the background using the Monte-Carlo method. Poor quality data sets that are not background-subtracted at this stage (i.e. sets where the discrimination between peaks and background is poor) can give rise to problems at the Pawley Fitting stage, due to correlation between the polynomial background and the very weak intensities.



- The pass filter value is set at 100 by default.
- Select **Preview** to run the fitting; the display now shows the background fitted as a green line. Check that it looks reasonable and is not removing any significant intensity from the peaks. A close-up view of a fitted background on a laboratory data set is given below:



- If you are not happy with the background you can try smaller or larger values of the *Low-pass filter window size*. This value is related to the number of data points per degree, and larger values tend to give a smoother, more featureless background, whilst smaller values allow the program to give a varying background more accurately. A closer look at the above example shows that whilst the background estimate is excellent there are a few ripples in the background line. Increasing the low-pass filter window size and applying it will smooth out these ripples. Select a value that suits your data, remembering to look closely at the high angle estimates, where the peaks are weak.
- Select **Accept** if satisfactory; DASH now subtracts the background from the data, as will be seen in the updated display.

2.5 Pawley-Fit Files

DASH keeps a record of progress while one is working on a chosen set of diffraction data. When you start a new project, the only item in the directory will be your diffraction data file. After indexing the cell, and extracting intensities by the Pawley fit procedure, DASH creates a file with extension `.sdi` that is then known as the Pawley-Fit file (see Section 2.5.1, page 11). This contains all the information needed for the last stage of the DASH process, i.e. structure solution using a molecular model.

The default name is based on the diffraction data file; e.g. for a compound called hydrochlorothiazide, we might have a data file *hct.xye*, producing a Pawley-Fit file *hct.sdi.* If you had a second set of experimental data for hydrochlorothiazide, e.g. *hctnew.xye* and chose this as the input file, DASH would create a new Pawley-Fit file called, by default, *hctnew.sdi*, but you can choose the names as you wish.

2.5.1 Pawley-Fit File: an Example

The Pawley-Fit file contains the full file names for various types of data that are created by DASH as the result of the Pawley refinement. It is not essential to know the details of these files, but it is important to be aware of the data files that are used by the structure solution process. Each line lists a file or some data that is identified by a keyword:

- **TIC**: Tick-mark file, with hkl and 2q positions of extracted intensities.
- **HCV**: Extracted hkl intensities and reflection correlations.
- **PIK**: Background-subtracted profile.
- **RAW**: File name for the original data file.
- **DSL**: Data type, wavelength, and peak shape parameters.
- **Cell**: the unit cell parameters.
- **SpaceGroup**: Space group selected for the Pawley refinement.
- **PawleyChiSq**: c^2 fit achieved by the Pawley refinement.

An example of the file *hct20.sdi* is given below:

```
TIC .\hct20.tic
HCV .\hct20.hcv
HCV .\hct20.hkl
PIK .\hct20.pik
RAW .\hct20.raw
DSL .\hct20.dsl
Cell    9.93861   8.49849   7.31737   90.0000  111.1896   90.0000
```

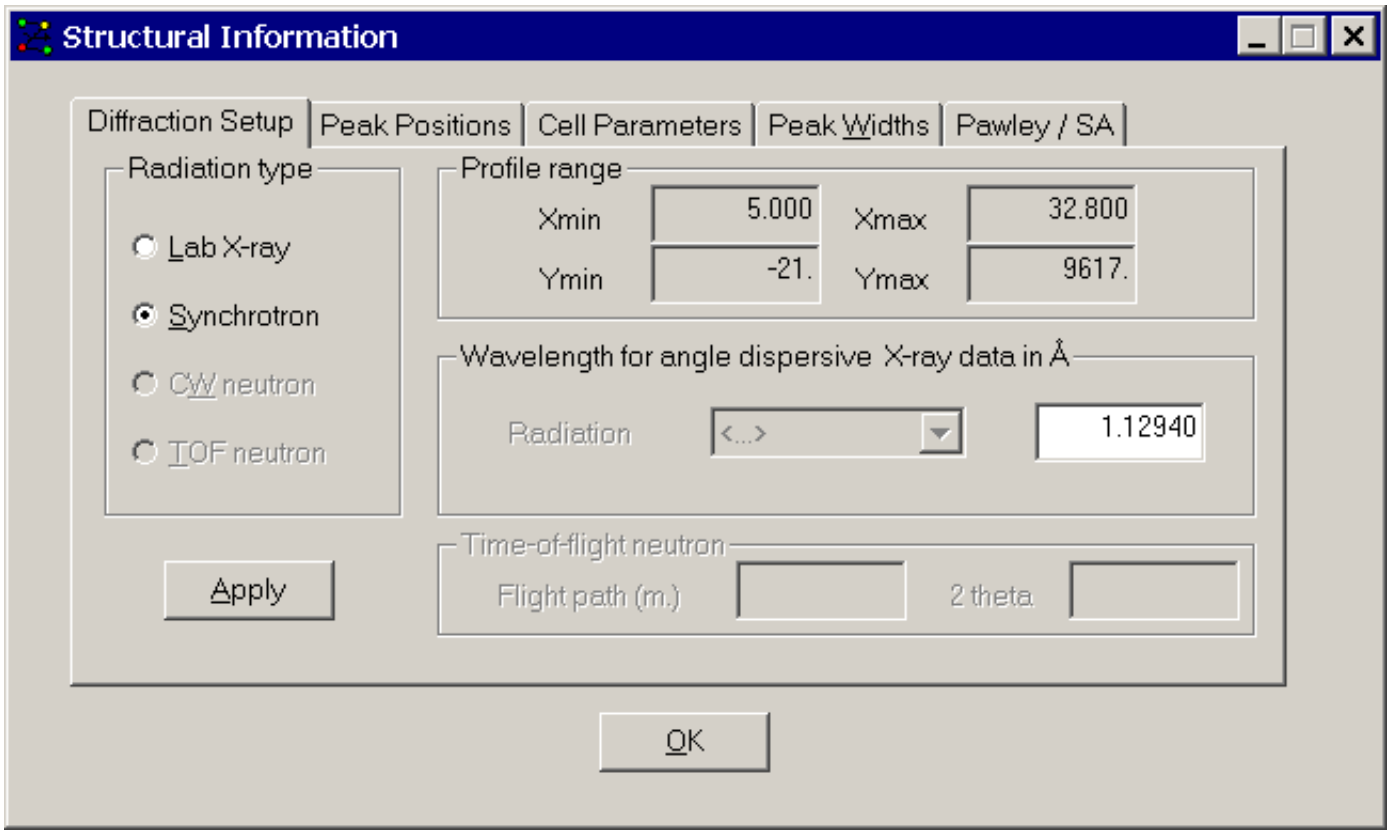
```
SpaceGroup   38      4:b       P 1 21 1
PawleyChiSq       2.41
```

2.6 Viewing Data Attributes, Peaks and Crystal Symmetry

After you have proceeded with the cell indexing step and choice of space group, there are options for viewing the current peak list etc. These items are selected from the top-level menu button **View**, as follows:

- **Diffraction Setup**: Type of data, wavelength etc. (see Section 2.6.1, page 12).
- **Peak Positions**: 2q for fitted peaks (see Section 2.6.2, page 13).
- **Cell Parameters**: Cell dimensions, space group etc. (see Section 2.6.3, page 14).
- **Peak Widths**: Parameters describing peak shape (see Section 2.6.4, page 15).

2.6.1 Viewing Diffraction Setup

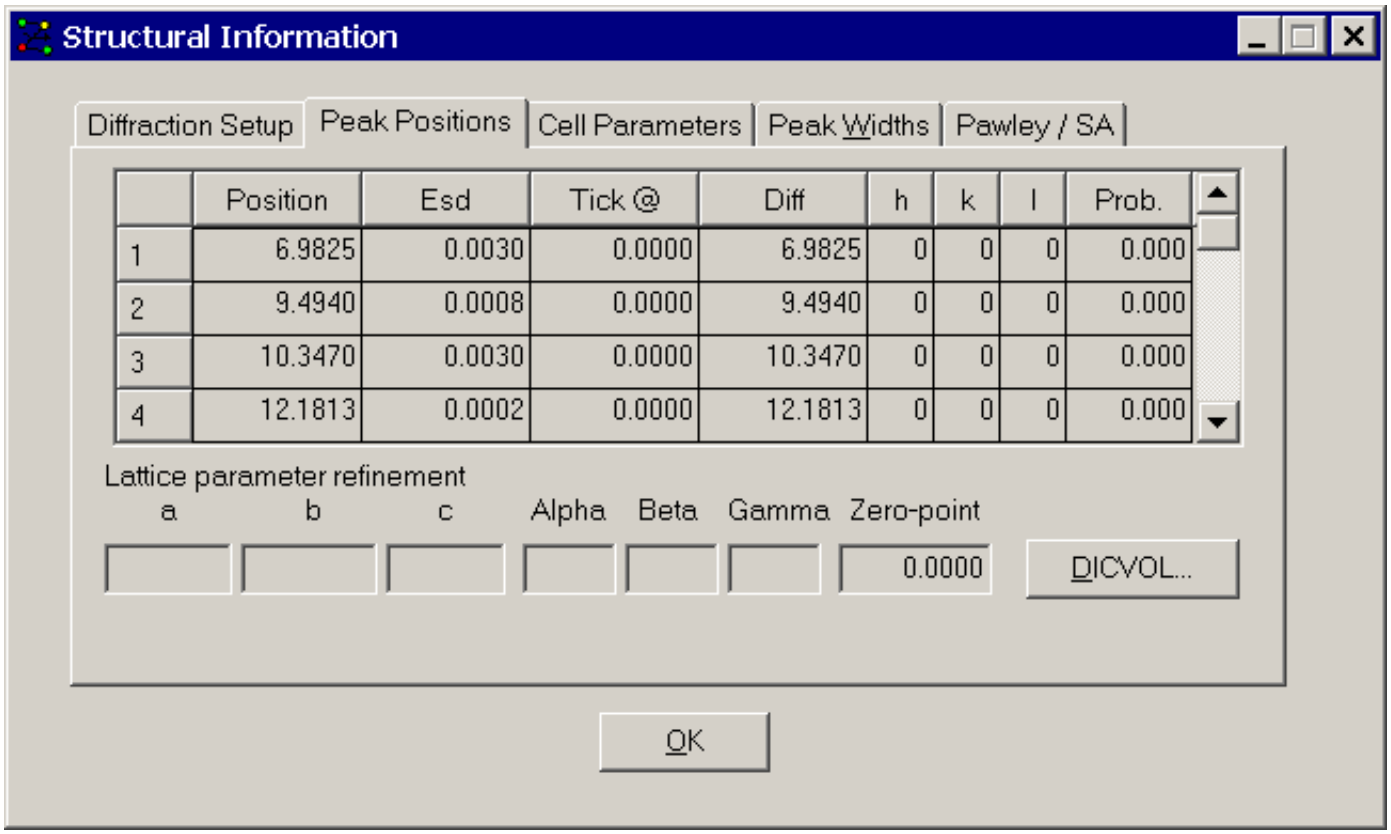


The above *Structural Information* window appears after selecting **Diffraction Setup** from the **View** menu. Information can be entered on:

- *Radiation type*: Laboratory or Synchrotron (DASH does not yet handle neutron data).
- *Profile Range*: this is for information only.

- *Wavelength*: the radiation wavelength used is entered here. For a synchrotron data set, you must enter the wavelength in the entry field. For laboratory data, you can either type the wavelength or use the pull down menu to select the appropriate radiation type.

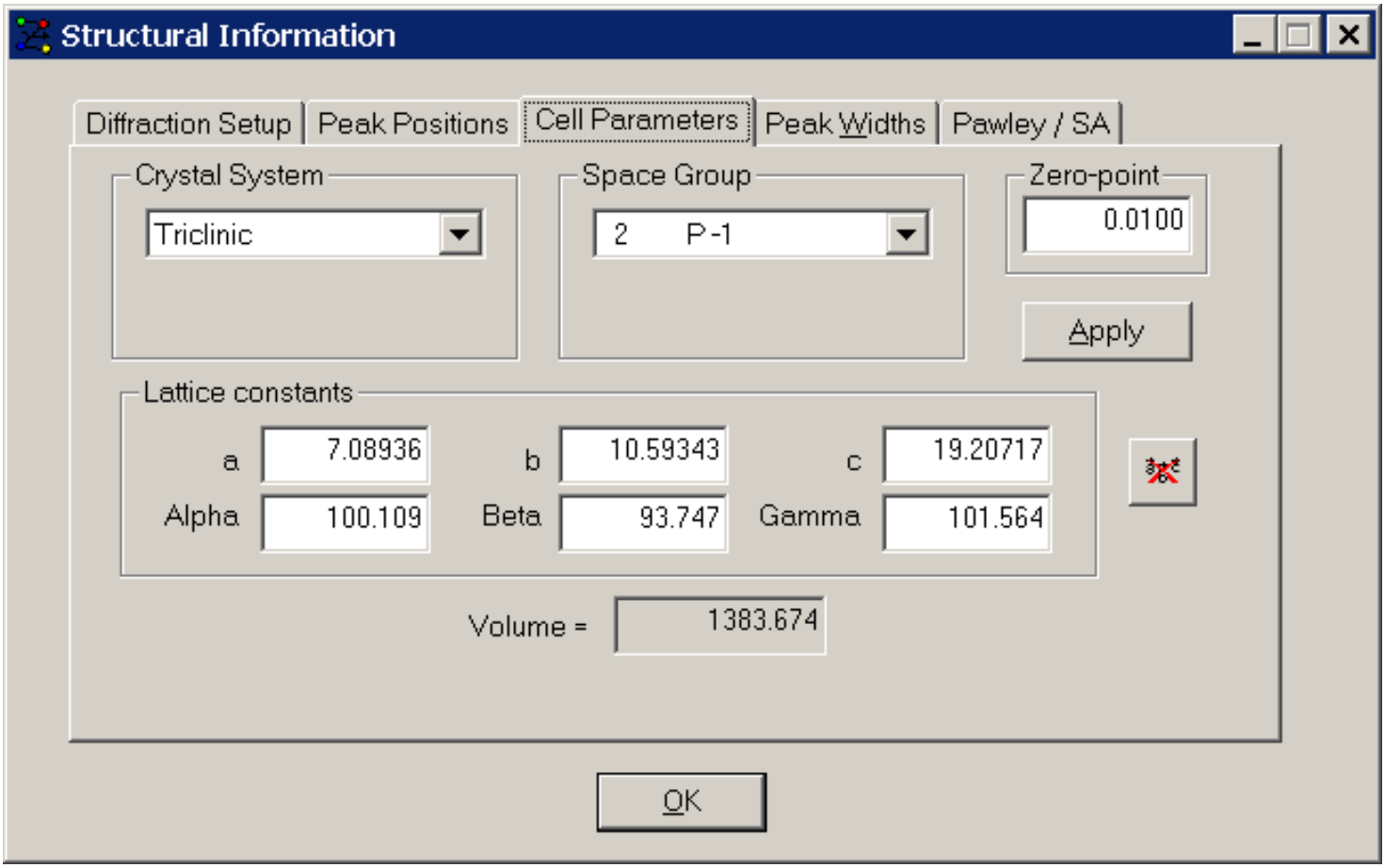
2.6.2 Viewing Peak Positions



The above *Structural Information* window appears after selecting **Peak Positions** from the **View** menu. This example shows a list of peaks fitted ready for cell indexing.

- *Position*: the peak position in 2q.
- *Esd:* the estimated standard deviation of the position in 2q.
- *Tick:* the 2q position calculated from unit cell (once entered).
- *Diff:* the difference between the observed position and the tick-mark.
- *hkl:* after unit cell indexing these are the Miller indices assigned to the peak.
- *Prob:* probability of correctness of assignment.
- *Lattice parameters* and *Zero-point:* these fields are filled in after a Pawley refinement, or when an *on-the-fly* cell refinement has been performed. These are display fields only.
- The **DICVOL...** button can be used to create an input file ready for the DICVOL indexing program, after fitting a number of peaks (see Section 6, page 55).

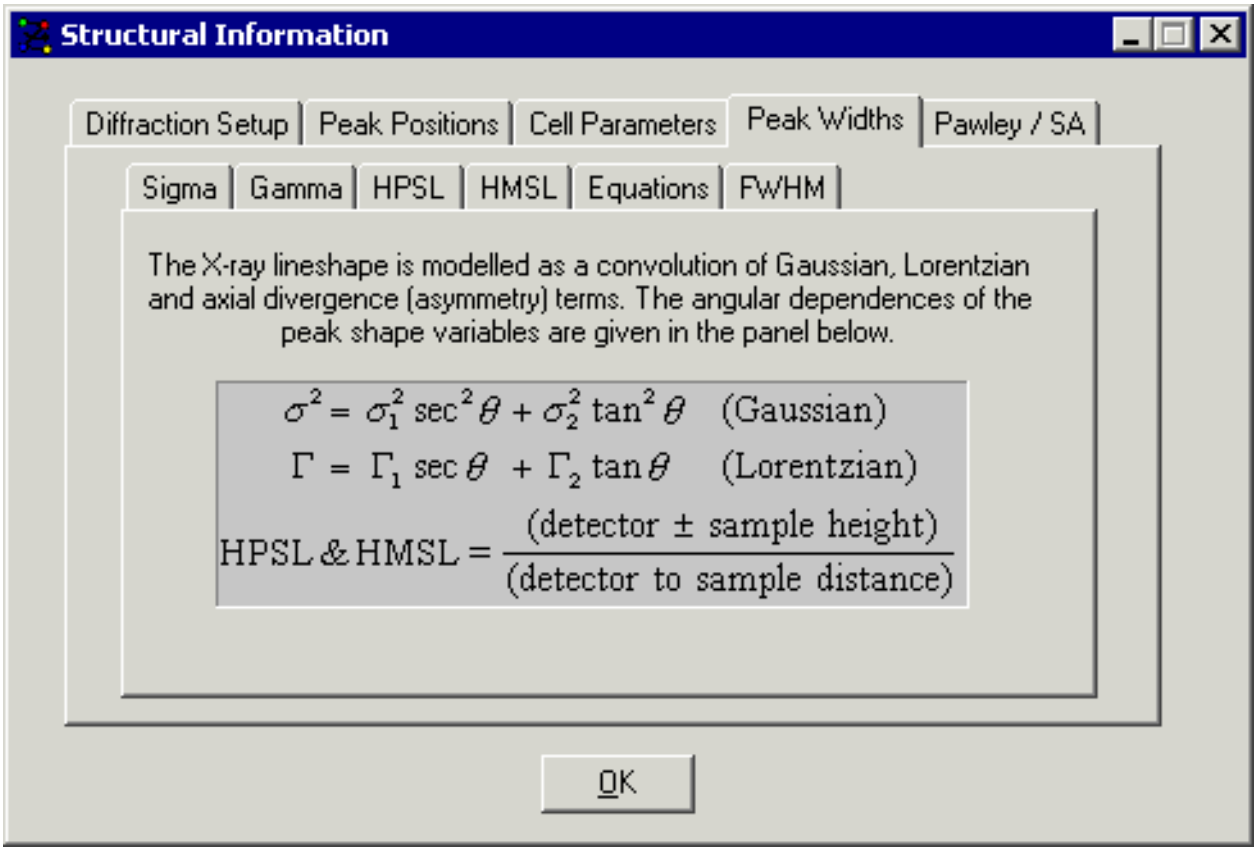
2.6.3 Viewing Unit Cell Parameters



The above *Structural Information* window appears after selecting **Cell Parameters** from the **View** menu. This example shows a cell and space group ready for input to the Pawley Refinement:

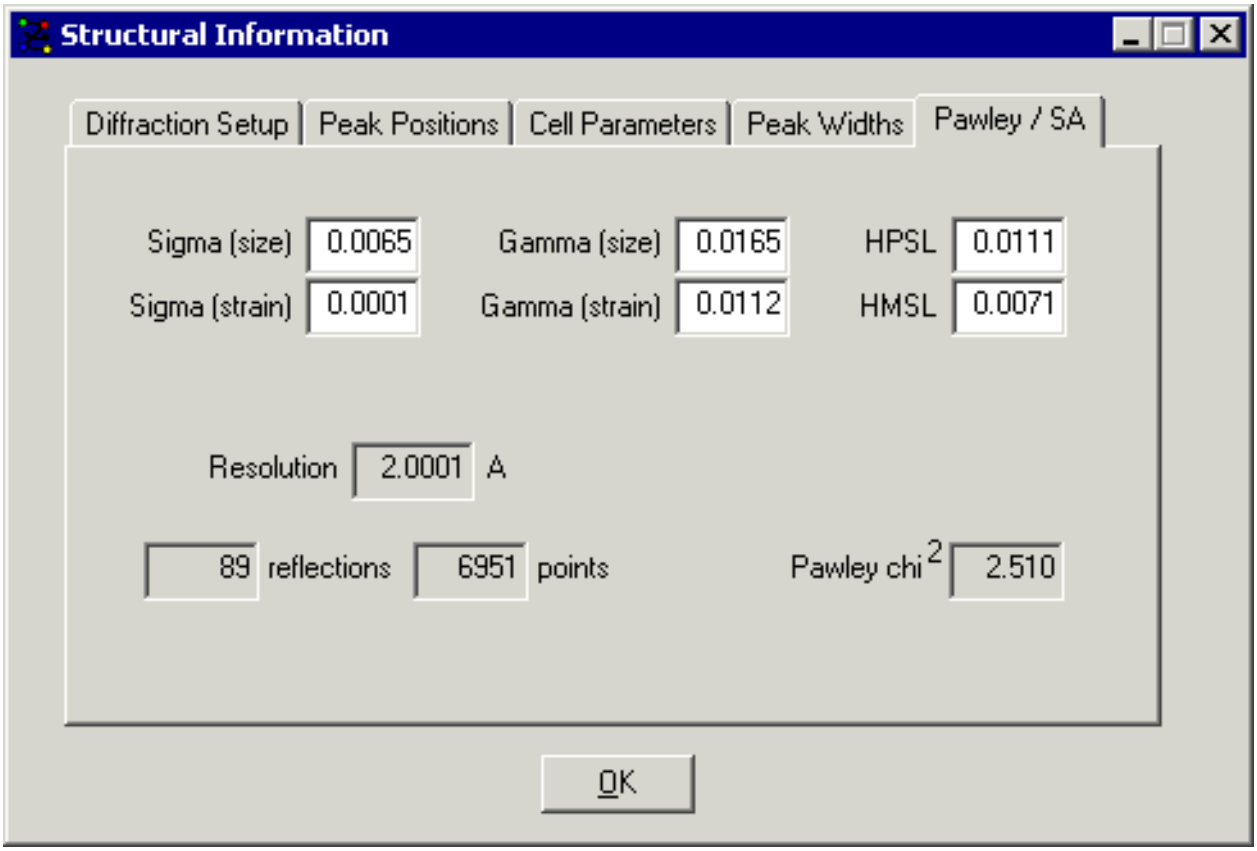
- *a, b, c*: unit cell lengths in units of Angstroms.
- *Alpha, beta, gamma*: unit cell angles (degrees).
- *Volume*: calculated volume of the unit cell (cubic Angstroms)
- *Zero-point*: zero-point error of the powder pattern in degrees 2 theta (if known).
- *Crystal System*: a list of the crystal systems.
- *Space Group*: a list of space group symbols in various settings.
- Clicking on the following icon clears the unit cell parameters:

2.6.4 Viewing Peak Widths



The above *Structural Information* window appears after selecting **Peak Widths** from the **View** menu. There are six tabs that allow you to access details of the peak description parameters. These fields are for display only. The fields are populated once a few peaks have been fitted, as reliable peak shape parameters have been calculated by this stage. Inspection of the values can be useful in deciding which peak shape parameters (if any) need to be varied in a Pawley refinement (see Section 8.6.2, page 85).

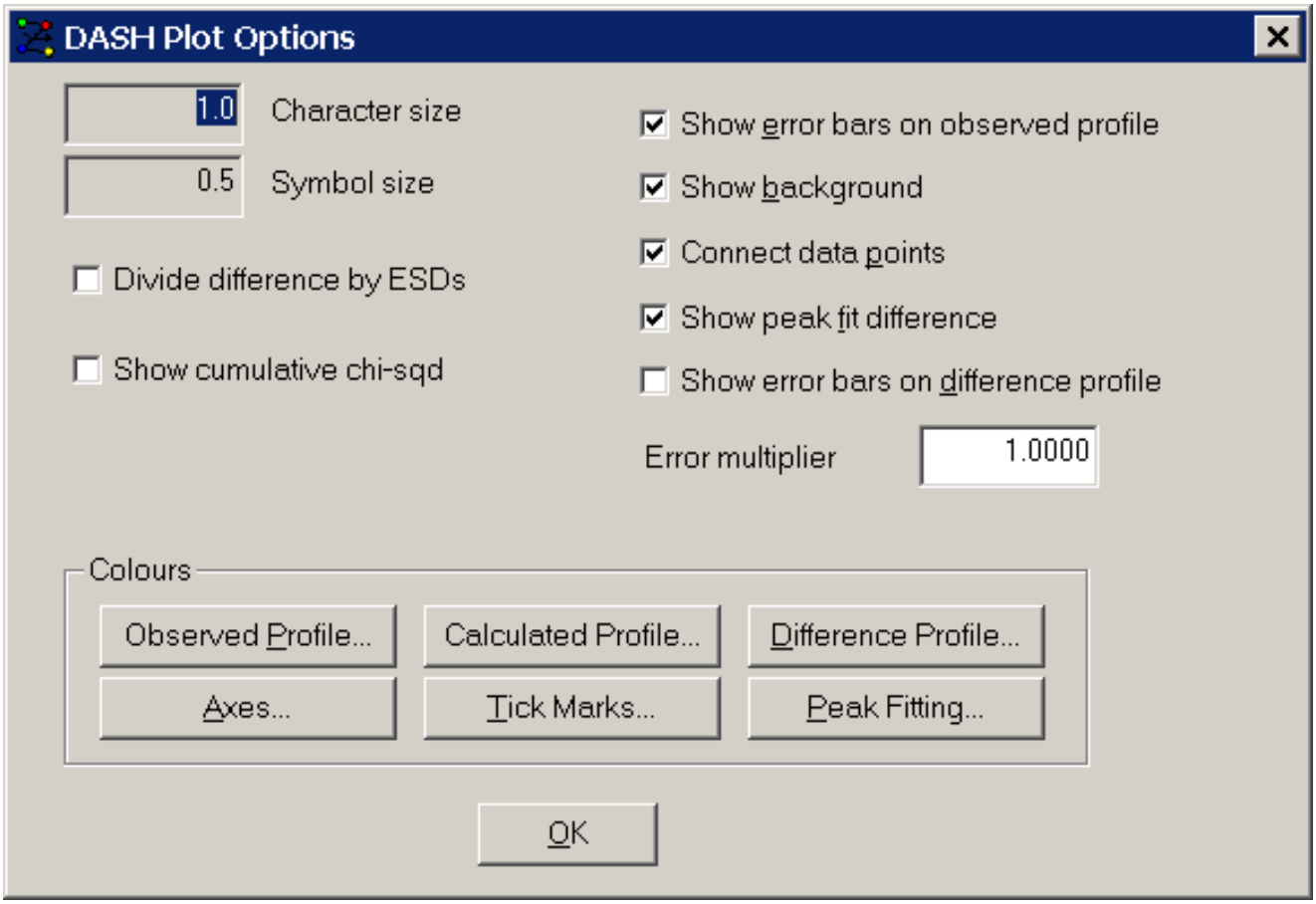
2.6.5 Viewing Pawley/SA



The above *Structural Information* window appears after selecting **Pawley / SA** from the **View** menu. This example shows several pieces of information about the last Pawley refinement. It is possible to manually enter values that will be used for subsequent Pawley refinements, but this should not usually be necessary.

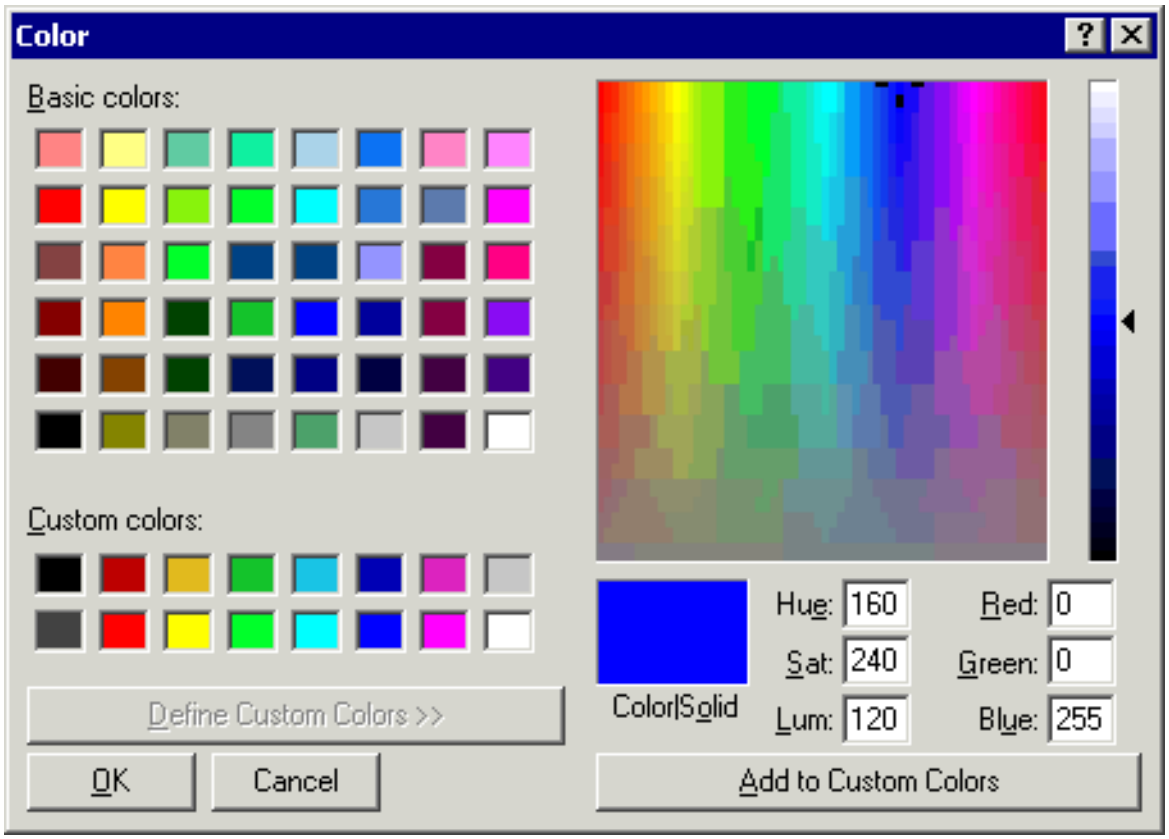
2.7 Plot Options for Graphics

The *DASH Plot Options* window appears after selecting **Options** from the top-level menu:

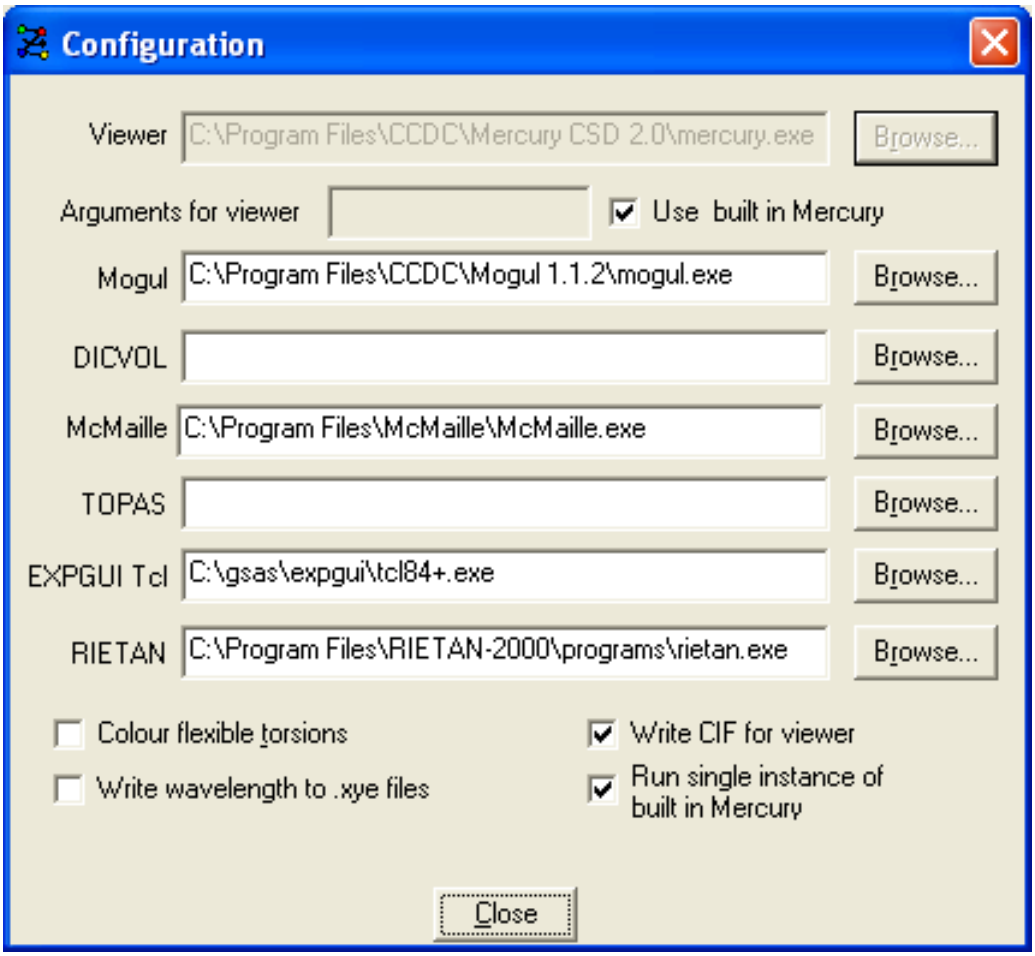


- *Character size*: not currently active.
- *Symbol size*: not currently active.
- **Show Error bars on Observed Profile:** display of the error bars on data point may be toggled on and off using this control.
- **Show Background:** display of the calculated background may be toggled on and off using this control.
- **Connect data points**: display of lines connecting the data points of the powder pattern may be toggled on and off using this control.
- **Show peak fit difference:** display of the difference between measured and fitted peaks may be toggled on and off using this control.
- **Show Error Bars on Difference Profile:** display of the error bars on a data point in the difference profile may be toggled on and off using this control.
- **Error Multiplier:** Used to control the value of the multiplier applied to error bars.
- **Divide difference by ESDs**: when ticked, the points of the difference curve are divided by the ESDs of the observed number of counts and multiplied by the average ESD.
- **Show cumulative chi-sqd:** display of the cumulative chi-sqd during Pawley refinement and simulated annealing may be toggled on and off using this control.

- **Colours:** these buttons may be used to alter the default colours used by DASH for display of the profile. Selecting any one of the buttons brings up a colour chooser, from which you may select a colour by clicking on the appropriate square:



2.8 Configuration



- **Viewer**: the viewer used for viewing Z-matrices and solutions. The viewer will display crystal structures from `.cif`, `.pdb` or `.mol2` files.
- **Arguments for viewer**: If using *built-in Mercury* the command line argument to `load-all-files` is supplied by default. This ensures that when `.cif` files are written for structures to be overlaid, all structures are loaded and displayed simultaneously. If *MercuryCSD* is used to view structures, supplying the command line argument `-client` will load each structure selected into a single instance of *MercuryCSD*. If, within *MercuryCSD*, the *Multiple Structures* check box is ticked, the structures will be displayed simultaneously.
- **Mogul**: If access to *Mogul* is available then enter the path to the Mogul executable here or click on the **Browse** button.
- **DICVOL**: If *DICVOL04* is available for indexing then enter the path to the DICVOL executable here or click on the **Browse** button.
- **McMaille**: If *McMaille* is available for indexing then enter the path to the McMaille executable here or click on the **Browse** button.
- **TOPAS**: If *TOPAS* is available for Rietveld refinement then enter the path to the TOPAS

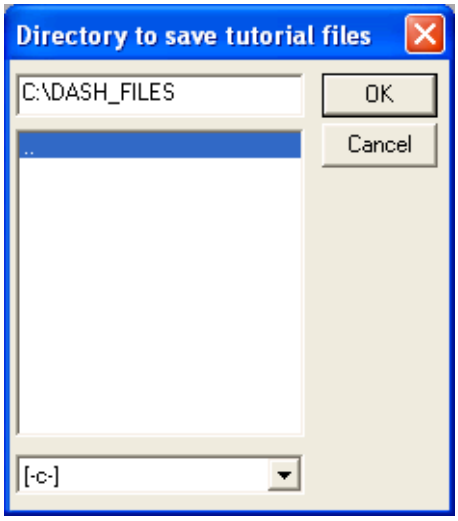
executable or click on the **Browse** button.

- **EXPGUI Tcl**: If *GSAS* is available for Rietveld refinement then enter the path to the `tcl84+.exe` (in the expgui folder) here or click on the **Browse** button.
- **RIETAN**: If *RIETAN* is available for Rietveld refinement then enter the path to the *RIETAN* executable here or click on the **Browse** button.
- **Colour flexible torsions**: when selected, flexible torsion angles are colour coded when viewing a Z-matrix.
- **Write wavelength to .xye files**: If checked the wavelength entered or selected when reading in a powder pattern will be automatically written to the `.xye` file.
- **Write CIF for Viewer**: If checked, individual `.cif` files will be written for all structures chosen to be overlaid. If using *built-in Mercury*, these files will be automatically displayed simultaneously. If *MercuryCSD* is used, the `.cif` files will be loaded but to display the structures simultaneously, the *Multiple Structures dialog* has to be invoked.
- **Run single instance of built-in Mercury**: When ticked, only one copy of *built-in Mercury* will be opened and all structures will be loaded into that instance of *Mercury*. Unticking the check box will cause a fresh copy of *built-in Mercury* to be opened each time a structure is selected for viewing.

2.9 Online Help

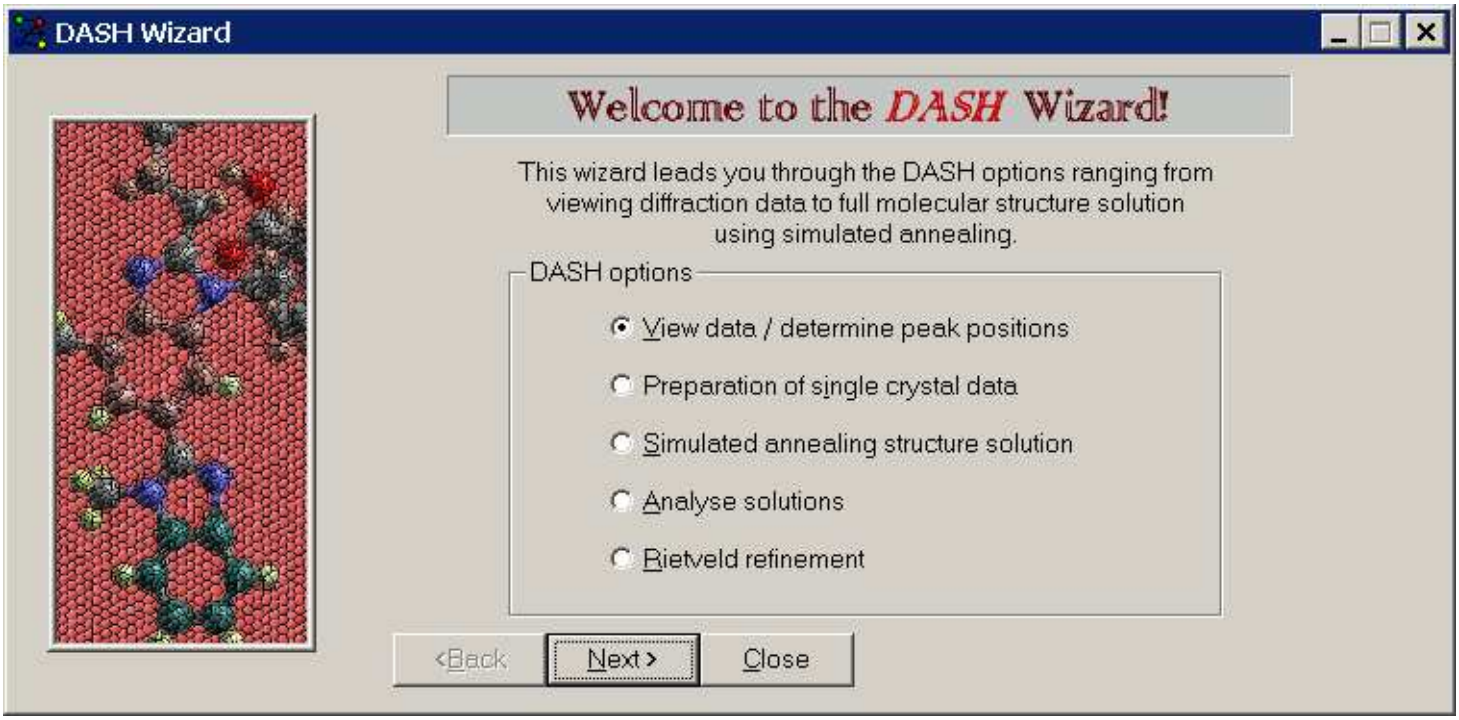
The online version of this user guide is available by clicking the **Help** button, the  icon or by using the keyboard command **Ctrl-H**. The following features are available:

- **DASH Help**: allows you to view a copy of the current user guide and also provides access to the Tutorials.
- **DASH Tutorials**: allows you to view a set of Tutorials for DASH. Clicking on any of the tutorials will cause a window to pop up prompting the user to supply a directory in which to save the files for the specific tutorial. Clicking on the ".." feature will cause the browser to go up a level within the directory structure for navigation purposes.



- **About DASH**: gives the DASH version number.

2.10 The DASH Wizard



The DASH Wizard has been designed to guide you through the structure solution process, which is performed in a series of steps:

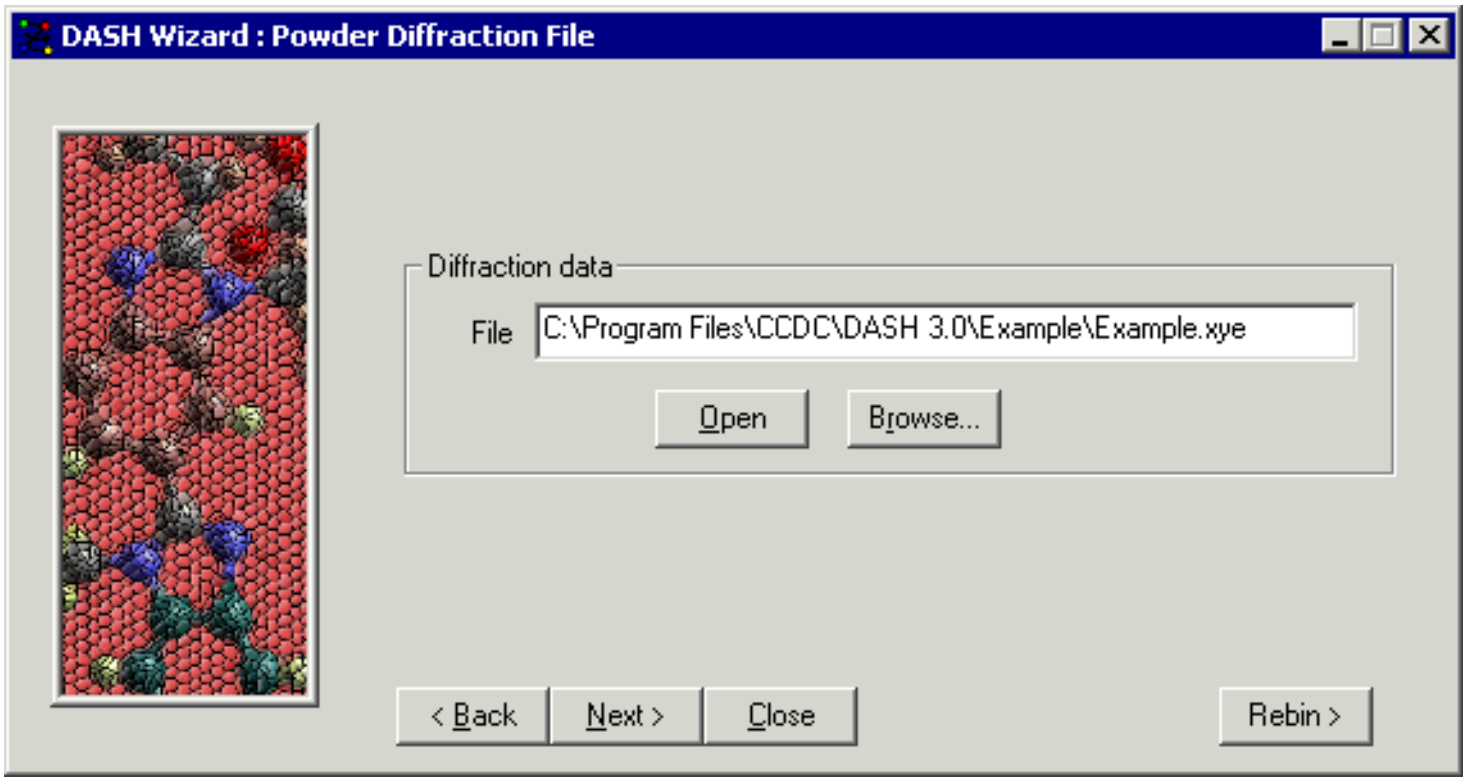
- **View data/determine peak positions** (see Section 2.10.1**, page 22)**
- **Preparation of single crystal data** (see Section 13**, page 153)**

- **Simulated annealing structure solution** (see Section 10**, page 101)**
- **Analyse solutions** (see Section 10.9.4**, page 129)**
- **Rietveld refinement** (see Section 12.1**, page 137)**

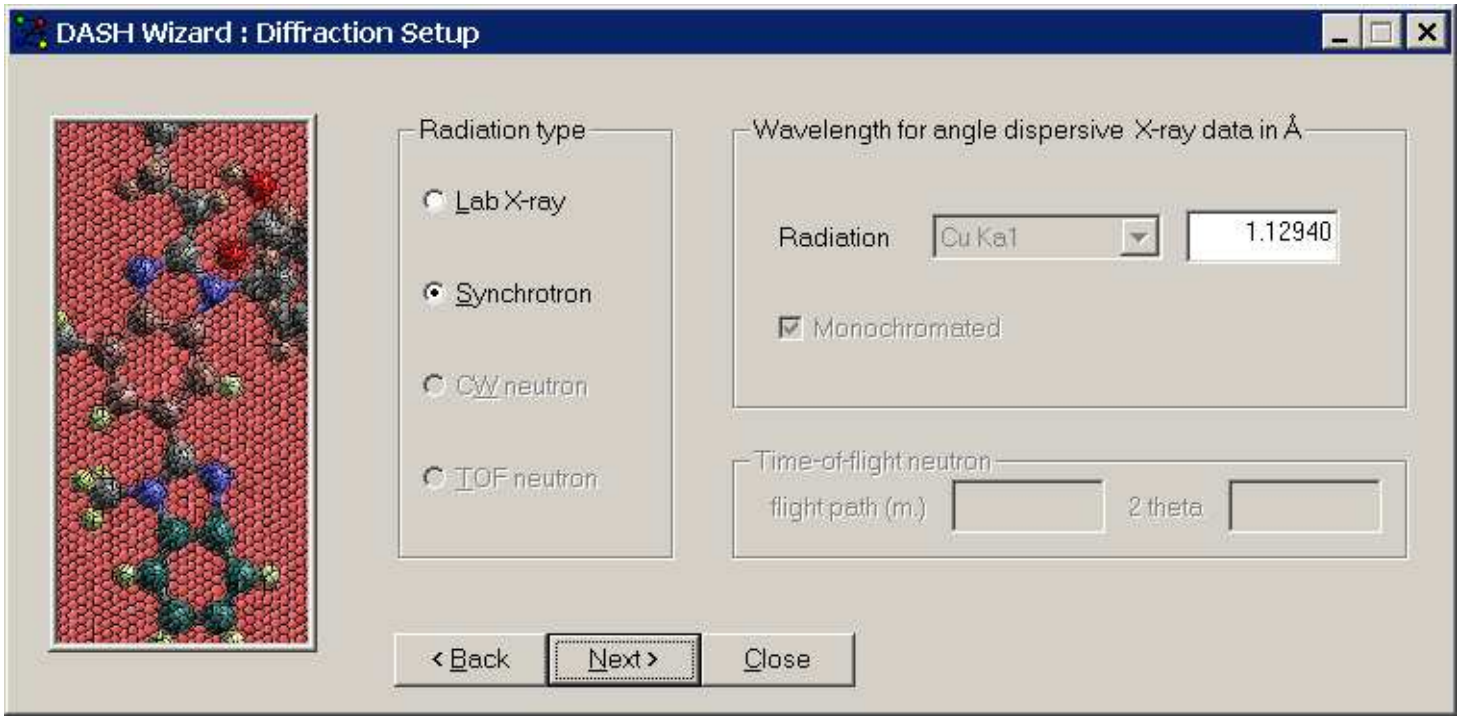
It may be called up at any time by clicking the  icon in the main window.

2.10.1 View Data / Determine Peak Positions

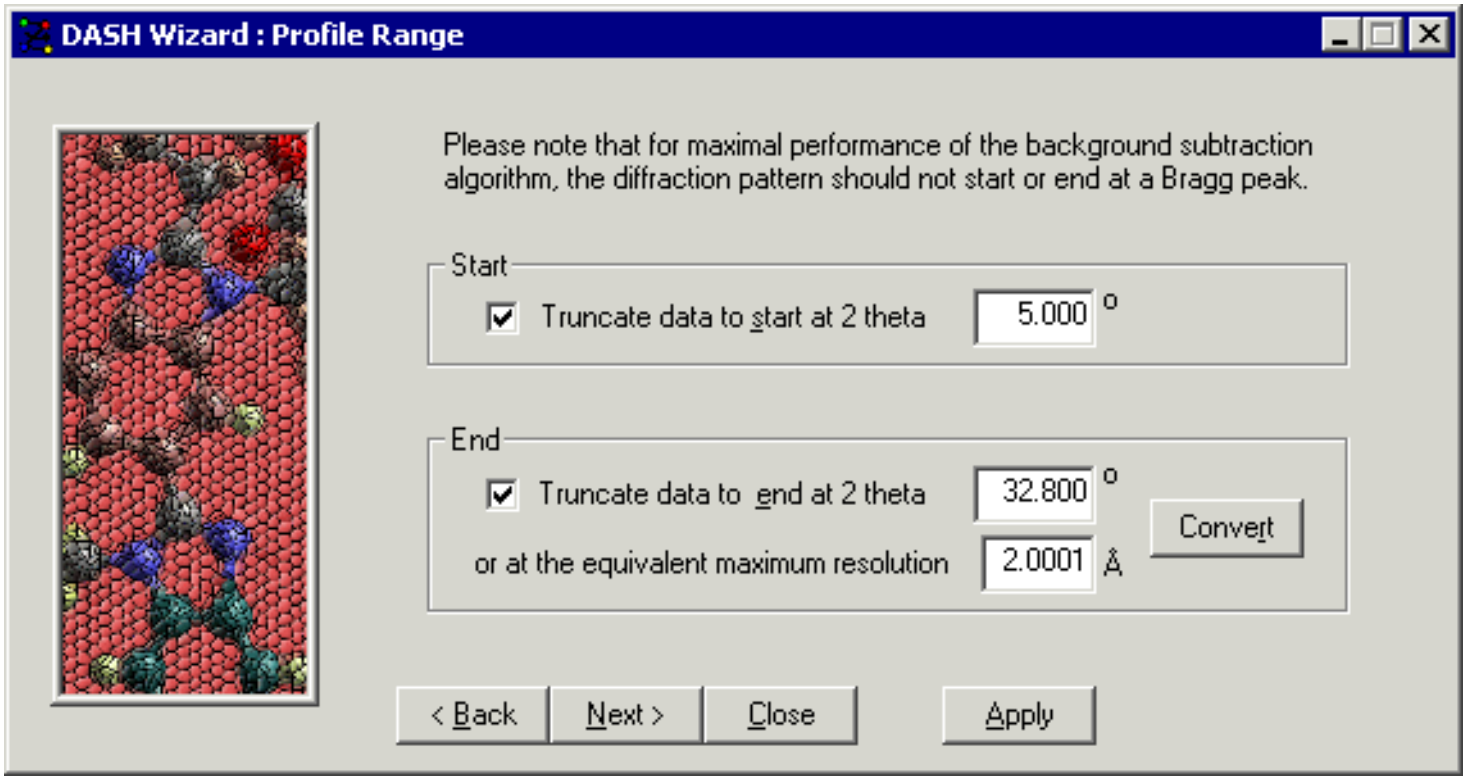
- Select the first option **View data / determine peak positions** and click **Next >**.



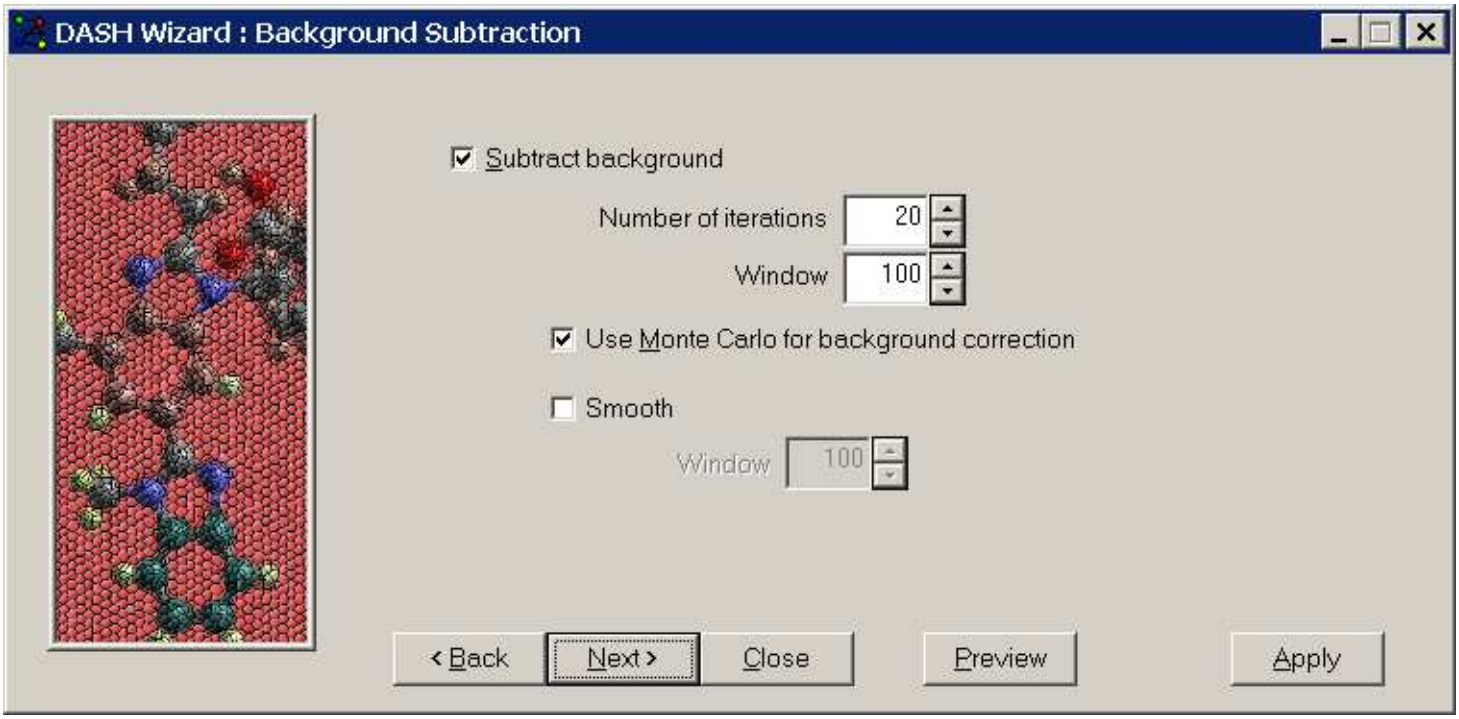
- Click **Browse...**
- Select a data file, e.g. *Example.xye,* and the diffraction data will be loaded into DASH and displayed.
- You are given the opportunity to rebin the data by choosing **Rebin >**, otherwise click **Next >.**



- Check that the radiation type and wavelength have been set correctly.
- Click **Next >**.

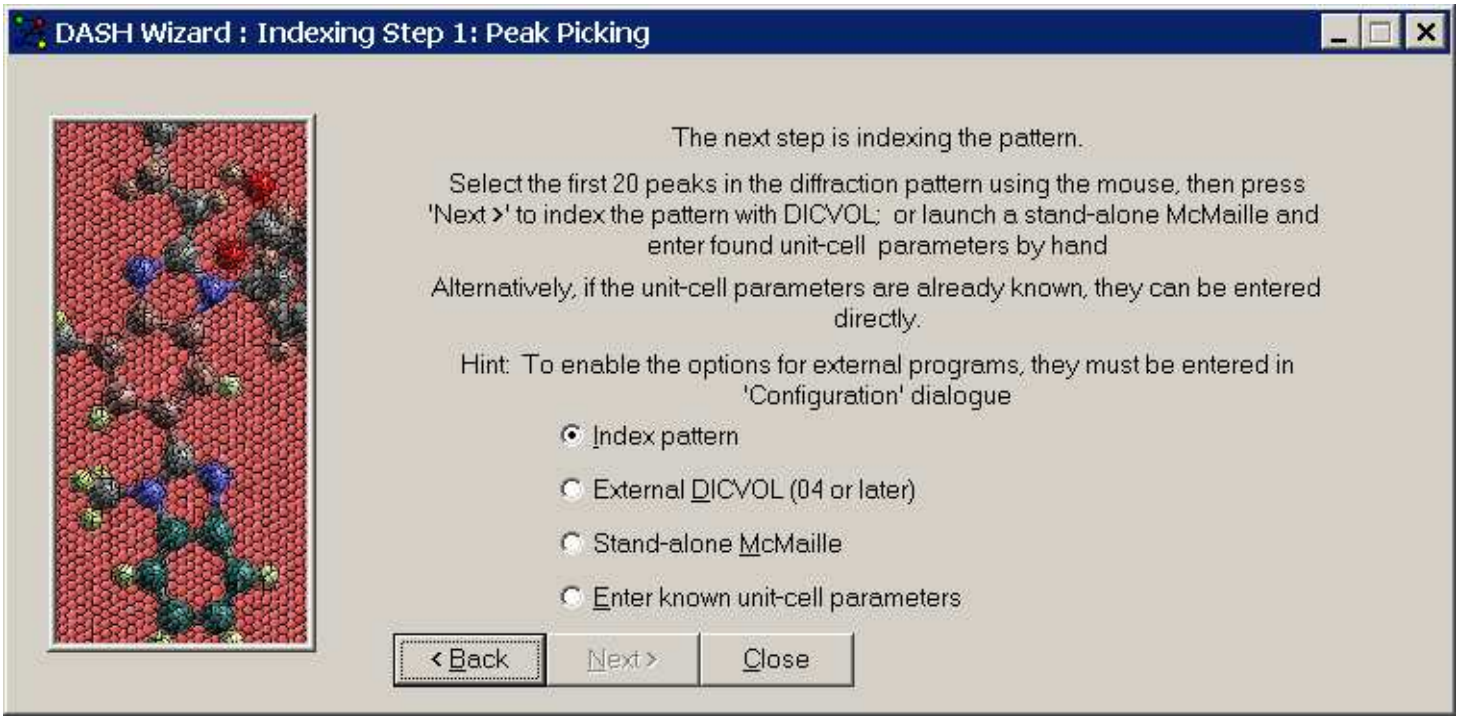


- Enter the 2q angle at which the pattern should be truncated at the beginning. This gives the possibility to remove the part of the pattern affected by the beam stop.
- Enter the 2q angle at which the pattern should be truncated at the end. This gives the possibility to truncate the data to a certain resolution (see Section 8.4, page 75).
- Click **Next >**.



- Adjust the width of the window to match the curvature of the background.
- Smooth: Checking this box will smooth the profile before the background is subtracted, using a window size of x data points, where x is specified in *Window*. The intensity at point `I` will be recalculated to be the average intensity of points `I-x` to `I+x`, where `x` is the window size.
- Click **Next >**.

2.10.2 Selecting an Indexing Package



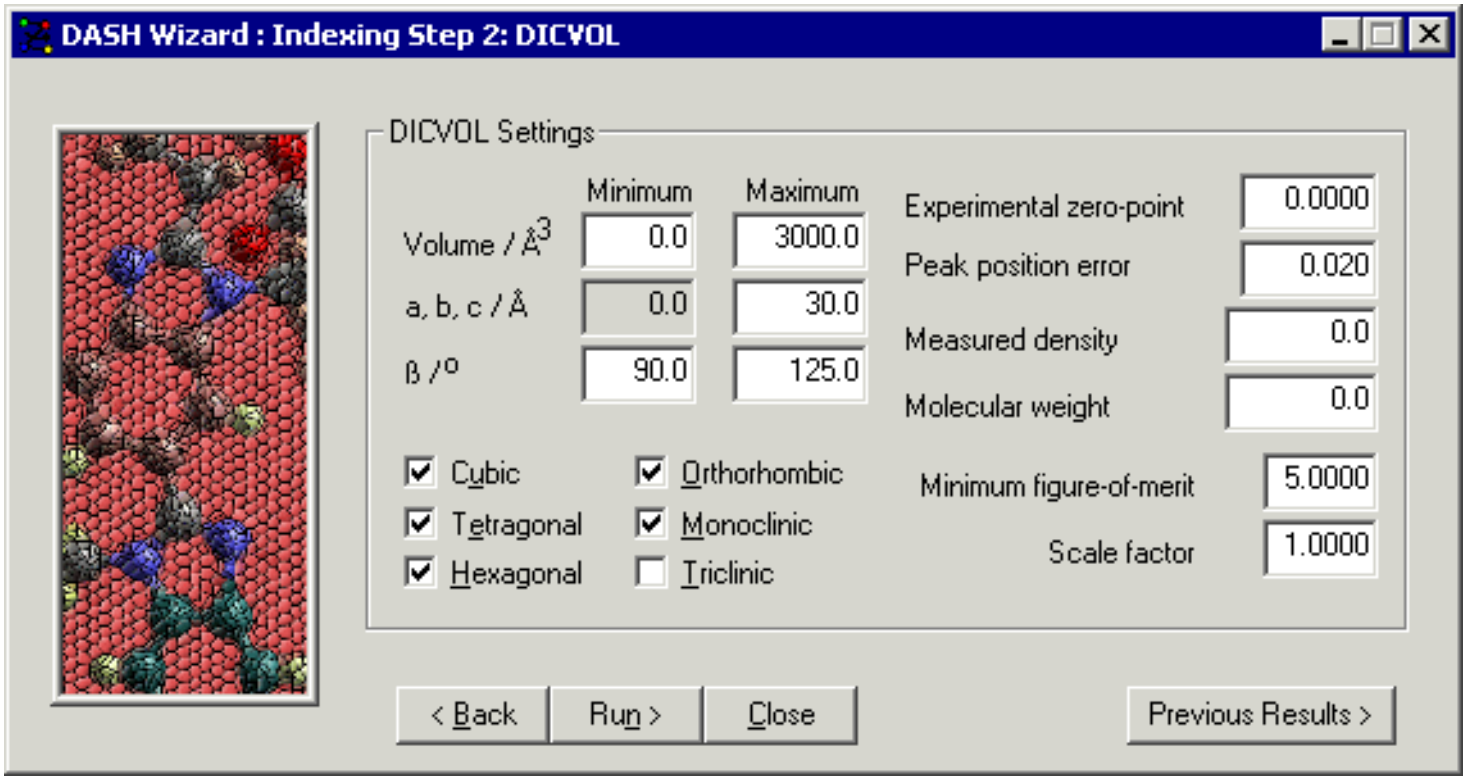
After you have picked some peaks for indexing:

- Selection of the check-box **Index pattern** will take you to an interface to DICVOL91.
- Selection of the check-box **External DICVOL (04 or later)** will take you to an interface to DICVOL04 (peaks from impurities allowed).
- Selection of the check-box **Stand-alone McMaille** will take you to an interface to McMaille.
- Click **Next>**

If you already know the cell parameters then:

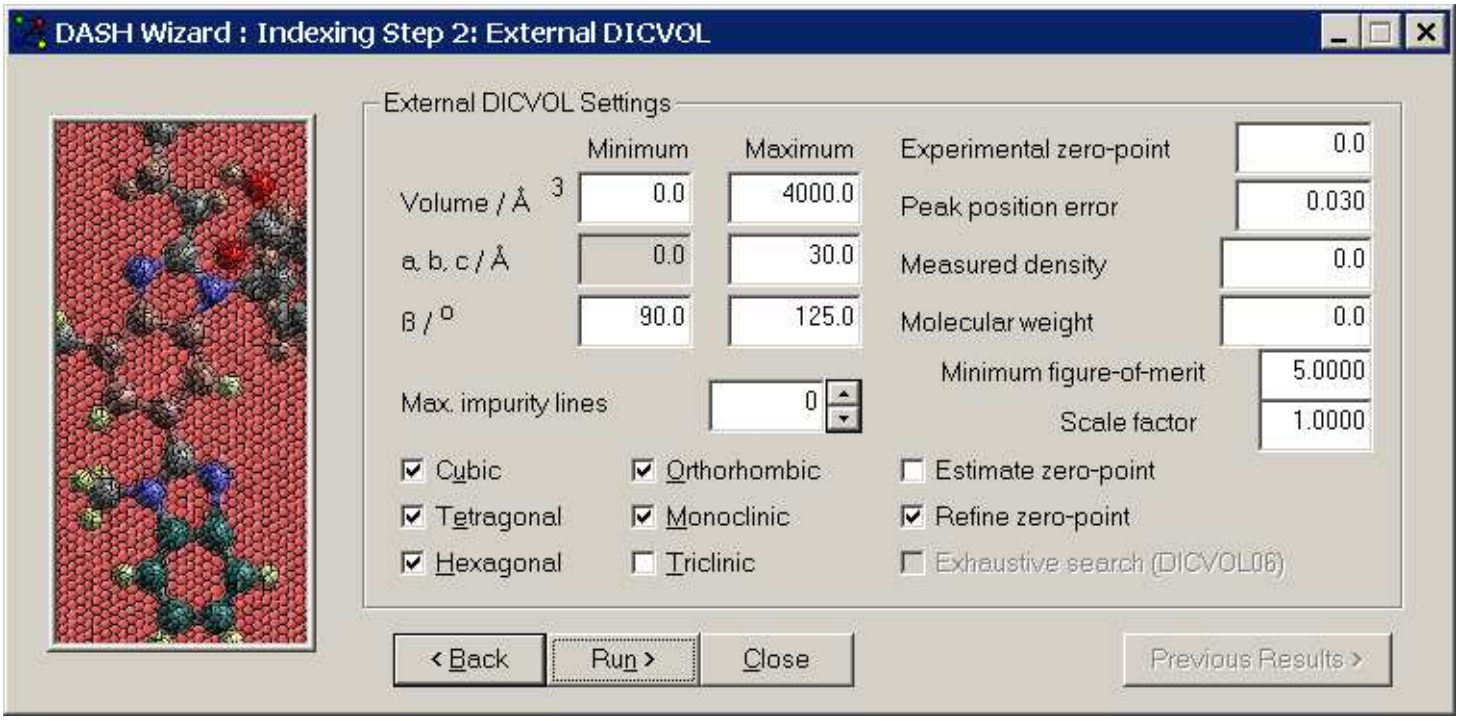
- Selection of the check-box **Enter known unit cell parameters** will take you to the Unit Cell Parameters window where the parameters can be entered.
- Click **Next >**.

2.10.3 Interface to DICVOL91



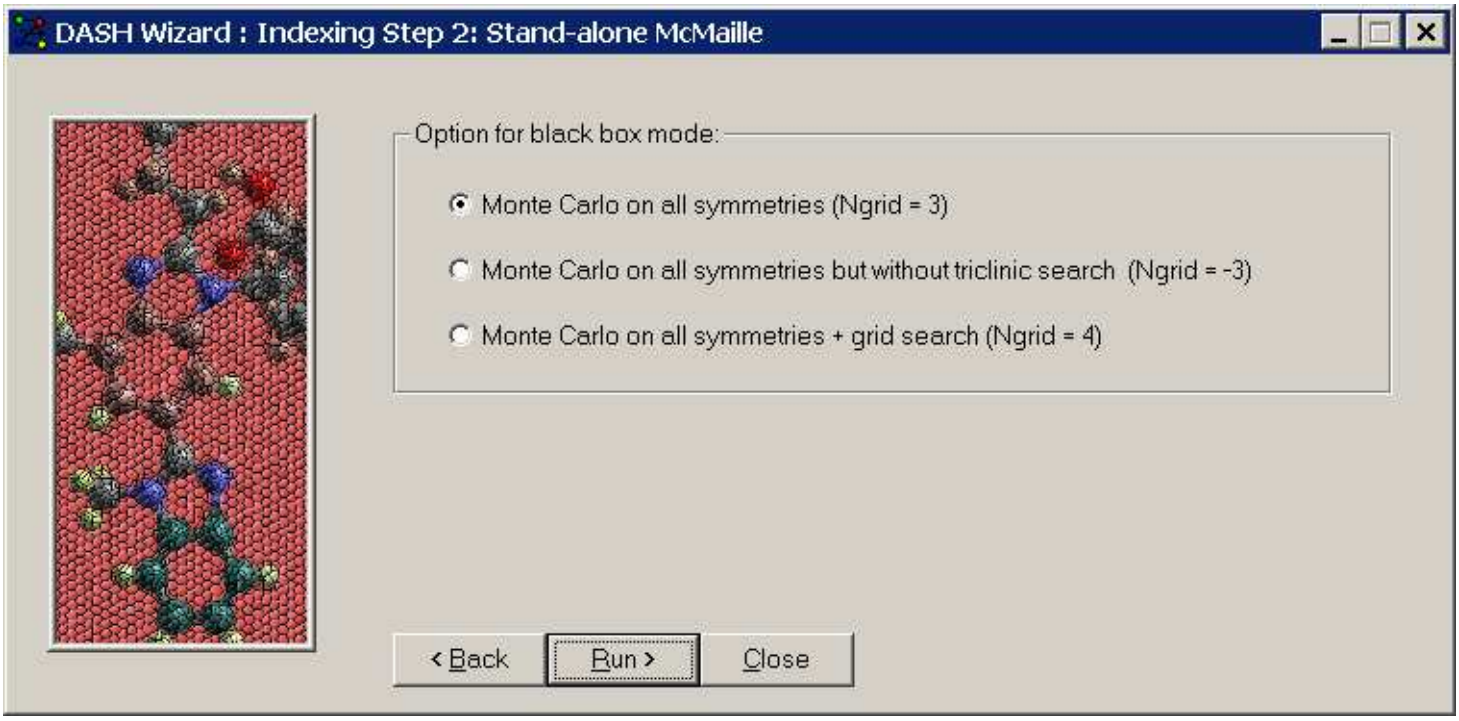
- If known, enter the experimental zero-point error.
- Select the appropriate crystal systems. Note that **Triclinic** might take a long time.
- Click **Run >**.
- Clicking on **Previous Results** will return you to the *Results* window, showing parameters obtained from a previous indexing.

2.10.4 Interface to DICVOL04



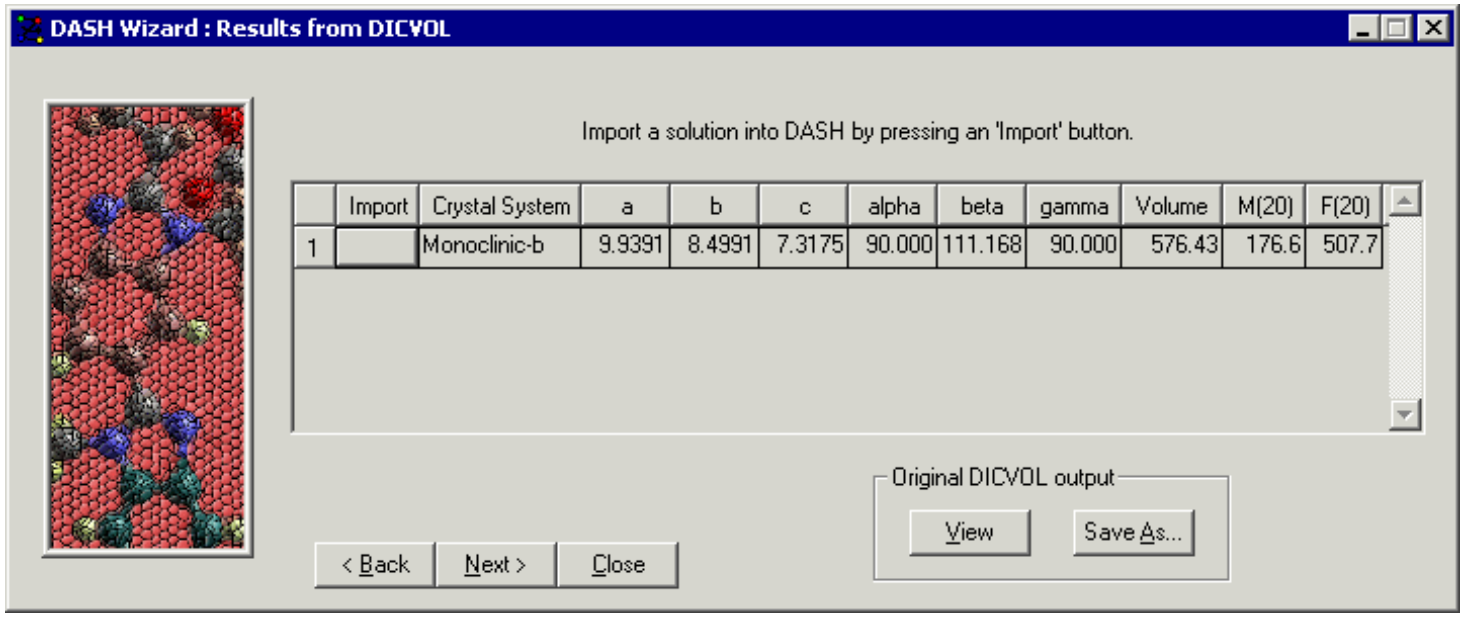
- If known, enter the experimental zero-point error.
- If known, enter the maximum number of impurity lines to be tolerated.
- Select the appropriate crystal systems. Note that **Triclinic** might take a long time.
- Click **Run >**.
- Clicking on **Previous Results** will return you to the *Results* window, showing parameters obtained from a previous indexing.

2.10.5 Interface to McMaille

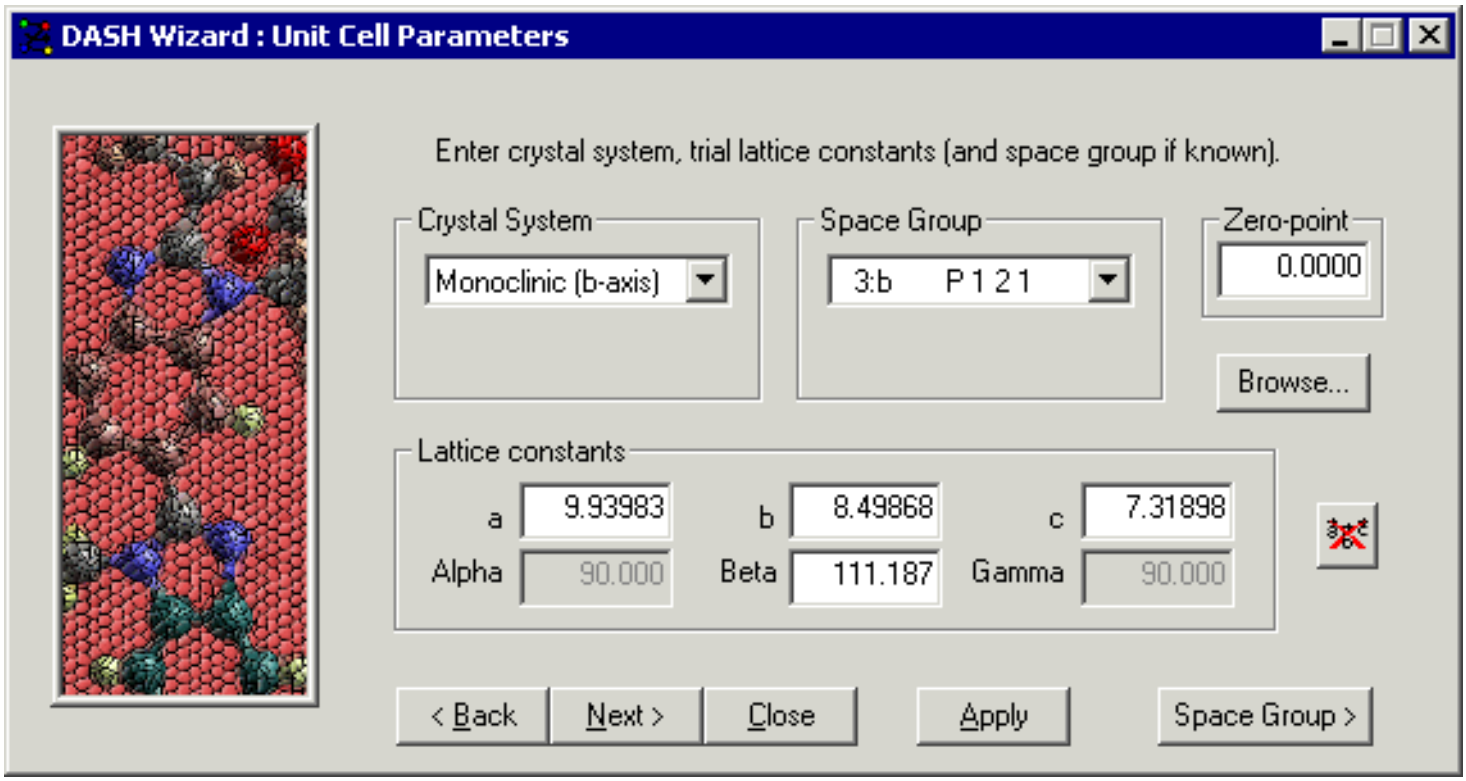


- Select which group of symmetries you would like to explore and whether a grid search is required.
- Indexing in the triclinic crystal system may take a long time with McMaille .
- Click **Run>**
- Once McMaille has finished running, you will be prompted to type a character and press return. The results from the indexing will then be presented in a text window. Closing this window will return you to DASH where you can enter your chosen cell parameters into the *Unit Cell Parameter* wizard window.(see Section 2.6.3, page 14)

2.10.6 Importing Unit Cell Parameters

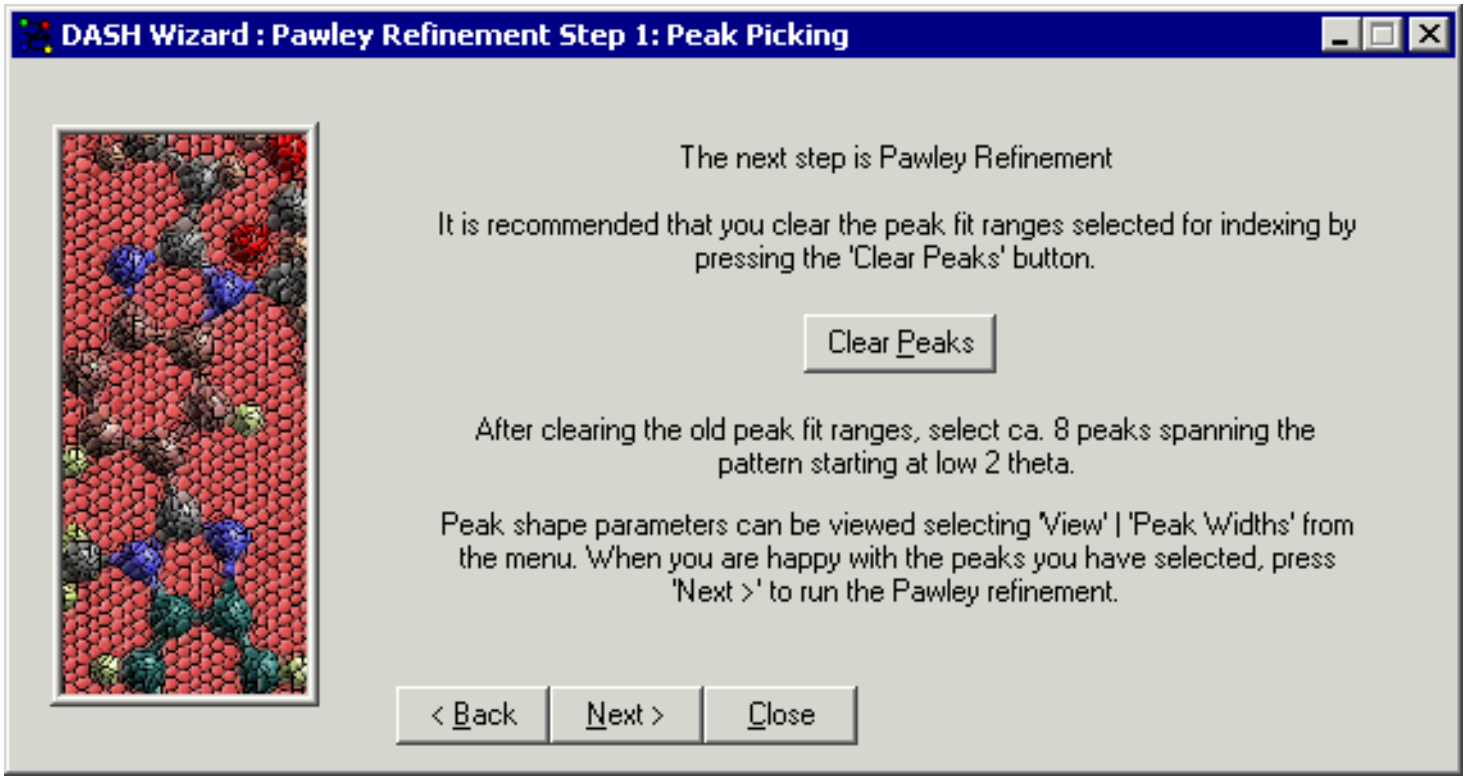


- Select the appropriate solution by selecting the button next to it in the *Import* column.
- Clicking **Next >** will take you automatically to the **Unit Cell Parameters** wizard window .



- Fill in details of the unit cell parameters and space group (see Section 2.6.3, page 14).

- The unit cell parameters can also be read in at this point from a crystal structure file by clicking on the **Browse...** button.
- Click **Next** >.



- Proceed with peak selection for indexing as described in Section 6.2, page 55.
- Click **Next** >.

Pawley refinement is the next step (see Section 8, page 69) followed by structure solution (see Section 10, page 101).

3 DATA COLLECTION AND DATA TREATMENT

3.1 Hints for Collecting Data

3.1.1 Synchrotron versus Laboratory Data

- DASH is capable of solving structures from both synchrotron and laboratory X-ray data.
- Synchrotron X-ray powder diffraction stations offer better instrumental resolution and positional accuracy, coupled with a vastly superior incident flux. These benefits manifest themselves best with nicely crystalline samples, where peaks that are overlapped in the laboratory X-ray pattern become well resolved in the synchrotron pattern.
- The collection of synchrotron X-ray powder diffraction data is indicated when laboratory data has failed to provide a solution, or when a precise high-resolution structure solution is required.

3.1.2 Choice of Detector Type

- Of the two common types of detector, scintillation detectors give better resolution but linear PSDs (position sensitive detectors) offer vastly better counting statistics. Use of either is likely to yield good results.
- If the sample line widths are well matched to the resolution of the PSD, there is little to be gained by switching to a scintillation detector.
- Most high-resolution powder diffractometers at synchrotrons currently use one or more scintillation detectors. Increasingly, though, image plates are being used to shorten data collection times and provide better counting statistics. The choice of which to use depends very much upon the prevailing instrumental set up; the station scientist is in the best position to advise you on such matters.

3.1.3 Required Resolution

- Global optimisation methods of structure solution do not require data collected to such a high angle as do direct methods of structure solution. Typically, if data can be collected to approximately 1.5 Å resolution, then structure solution will be feasible (Note that we are speaking here of spatial resolution within the data set). The 2q value corresponding to this resolution can be easily calculated from:

$$2q_{1.5Å} = 2*\sin^{-1}(l \ / 3.0 \text{ Å})$$

- Data should be collected to as low a 2q value as possible since the low-resolution reflections help with the indexing process. Generally, this low angle limit is imposed by the diffractometer, because there is a risk of damage to the detector from the straight-through beam at 2q values close to zero.

3.1.4 Obtaining Monochromatic Radiation in the Laboratory

- The radiation source should ideally be monochromatic.
- In decreasing order of preference, monochromatisation can be achieved by:
  - Use of a primary monochromator, i.e. one that lies between the X-ray tube and the sample. For a copper X-ray tube, this eliminates CuK_{a2} and CuK_{b}, leaving only CuK_{a1}.
  - Use of a secondary monochromator, i.e. one that lies between the sample and the detector. These usually eliminate only CuK_{b}, leaving both CuK_{a1} and CuK_{a2}. The CuK_{a2} contribution then needs to be stripped out algorithmically, which is a complication best avoided.
  - The use of filters to achieve monochromatisation is considered to be inappropriate for structure solution work.

3.1.5 Choice of Wavelength

- It is doubtful if any particular wavelength offers an advantage when dealing with organic powder samples. Whilst longer wavelengths spread out the pattern and would seem to decrease the chances of peak overlap, the peaks themselves widen and thus no advantage is gained.
- In situations where it is possible to select a wavelength (e.g. at a synchrotron), it should be chosen to maximise the incident flux, unless compelling reasons (such as absorption) dictate otherwise.

3.1.6 Data Collection Geometry

- Transmission geometry is recommended, with the sample in a rotating capillary.
- It is also possible to collect diffraction data in transmission mode when the sample is held as a thin film in a suitable attachment. Reflection geometry may be used, but there is a high risk of preferred orientation having a significant impact on the diffraction pattern. Whilst a small degree of preferred orientation can be tolerated in a structure solution, it is a complication that is best avoided.

3.1.7 Background Reduction

You should endeavour not to introduce any additional background scattering. For example, it may be appropriate to use borosilicate capillaries rather than glass or quartz, in order to avoid seeing the amorphous scattering from the capillary manifest itself as a background *hump*.

3.1.8 Zero-Point Calibration

Although it is possible to refine instrumental zero-point errors in the whole pattern fitting stage, it is always preferable to calibrate the instrument prior to a structure solution using a well defined standard sample, e.g. NBS silicon.

3.1.9 One Long Scan versus Several Short Scans

It is generally better to perform several short scans and sum them together using the data reduction software, rather than collecting a single long run. For example, four one-hour runs are preferable to one four-hour run. Prepare each sample fresh in order to reduce preferred orientation.

3.1.10 Choice of Step Size

- Ideally, you should have plenty of points across every peak in the diffraction pattern in order to accurately describe the underlying peak shape.
- If you use a step size that is too large, you risk missing subtle features such as peak shoulders that may be critical at the indexing stage.
- If there is any doubt, it is better to collect data on a finer grid, since a coarser grid can always be constructed later by re-binning the data; the converse is obviously not true.

3.1.11 How Long to Count For

- Obviously, the longer the time spent on collecting data, the more closely it will resemble the *true* diffraction pattern.
- For each doubling of the collection time, the estimated standard deviations are improved by a factor of $2^{1/2}$. Eventually, a stage is reached where substantial increases in collection times are required in order to achieve modest improvements in the signal-to-noise ratio.

- As a general guide, the data should be collected sufficiently long that reflections can be clearly distinguished from the background at around 1.5Å spatial resolution. Not all samples will diffract strongly to this resolution, but it remains a useful rule of thumb.

3.1.12 Optimising Use of Data Collection Time

- For a given data collection time, the question arises of how to optimise the use of that time. A common formula is:

$$\textit{Time per step} = (\textit{expt duration in seconds}) \,/\, ((2\theta_{max} - 2\theta_{min}) \,/\, \textit{step size})$$

- This scheme gives equal weighting to all data points and takes no account of the fact that the diffracted intensities at low angle will be much stronger than those at high angle. In many cases, this may be sufficient, but a more sophisticated data collection strategy is described in *J. Mater. Chem.* (1997) **7**, 569-572. Implementing such a data collection scheme is a simple matter and is strongly recommended when using scintillator detectors on a laboratory or a synchrotron source.

3.1.13 Neutron Data

DASH does not currently handle neutron diffraction data.

3.2 Hints for Treating Data

3.2.1 Lorentz Correction

DASH always corrects the data for Lorentz effects, so no correction should be applied to the data in advance.

3.2.2 Polarisation Correction

- In the case of synchrotron data, DASH assumes that the incident radiation is vertically polarised and, provided that the **Synchrotron** radiation option is turned on when the radiation wavelength is entered, no pre-processing of the data is necessary.
- In the case of laboratory X-ray data, DASH applies an appropriate polarisation correction provided that the **Laboratory** radiation option is turned on when the radiation wavelength is entered, and no pre-processing of the data is necessary.

- The exact form of the polarisation correction applied is suitable for instruments equipped with a primary monochromator. Whilst not exactly correct for different instrumental geometries, it is still a good enough approximation to be useful.
- It is always possible to fully correct for the polarisation effects of particular geometries, if your data processing software allows it, before inputting the corrected data into DASH. Within DASH, the data should then be treated as having been obtained using monochromatic synchrotron radiation.
- A Lorentz correction should *never* be applied in advance.

3.2.3 K_{a2} Stripping

- DASH is able to handle data collected using monochromatic radiation only. The use of more than one incident wavelength is a serious complication that should be avoided when tackling problems of structure solution.
- However, it is possible that your diffractometer software may provide suitable K_{a2} stripping routines that allow you to export a data file from which the K_{a2} contribution has been removed algorithmically. In such cases, the exported file may be treated within DASH as a monochromatic laboratory X-ray data set.
- NB: Stripping algorithms inevitably introduce some degree of uncertainty into the data.

3.2.4 Esds

- Each (2q, count) data point must be accompanied by an estimated standard deviation (esd). Ideally the diffraction data set input into DASH should consist of three columns of data:

  <2q> <*count*> <*estimated standard deviation*>

- Many diffractometers will output such a listing. However, if only

  <2q> <*count*>

  are available in the input file, DASH will automatically calculate the esd values from counting statistics.

3.2.5 Background Subtraction

- Some diffractometer software may offer the possibility of background subtraction. However, it is better to leave modelling of the background to DASH, unless there is a good reason to do otherwise e.g. if you have an appropriate physical model for the background and therefore can remove the background with confidence.
- DASH provides a robust Monte-Carlo background fitting option that is recommended for use with most data sets.

3.3 Checklist for Diffraction Data

- Use synchrotron or monochromatic laboratory X-ray radiation.
- If possible, collect data to at least 1.5 Å resolution.
- Use transmission capillary geometry.
- Do not apply Lorentz or polarisation corrections, or subtract the background, before entering DASH. DASH will assume raw data and perform these steps itself.
- Esds are preferable in the input file i.e.

  <2q> <*count*> <*estimated standard deviation*>

  If only <2q> <*count*> is available, DASH will automatically calculate esds.

4 PRELIMINARY INSPECTION OF PROFILE

Unfortunately, it is not possible to guess whether a structure will solve just by looking at the diffraction data. However, a preliminary visual inspection of the data is always worthwhile, as it may give clues about possible problems. Things to look out for are:

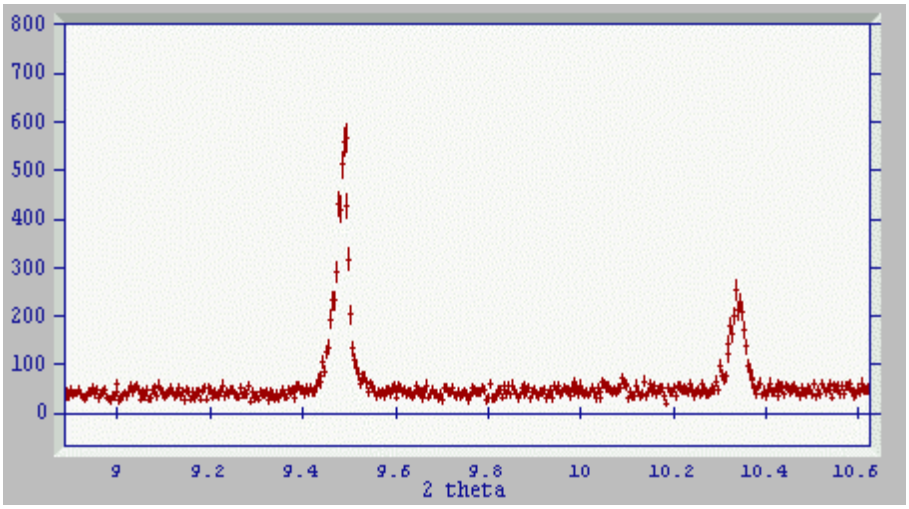
- Signal-to-noise ratio (see Section 4.2, page 38).
- Esds (see Section 4.3, page 42).
- Background shape (see Section 4.4, page 42).
- Peak shapes (see Section 4.5, page 43).
- Balance of peak intensities (see Section 4.6, page 44) and (see Section 4.7, page 45).
- Useful 2q range (see Section 4.8, page 45).

4.1 How to Use the Interface to Inspect a Profile

When you input a diffraction data file to DASH, the default display is of the complete data set, over the full range of 2q. There are several methods for examining chosen areas of the data set.

4.1.1 How to Zoom in to a Chosen Area

- The simplest way to zoom is to use the left mouse button; ensure that you are in Zoom mode (this is the default mode) by selecting **Default** from the **Mode** menu, or depressing the icon on the menu bar.
- Click and hold the left mouse button and drag out a rectangle around the area that you want to zoom in on.
- To zoom out, simply select the **Home** key on the keyboard. This example shows the effect of zooming in on two peaks that lie just either size of 10° 2q. You will see that DASH plots both the intensity and the error bars.



- A useful keyboard short-cut for zooming in on the 2q axis is to select **Shift -**å. Selecting **Shift -**é will zoom out on the 2q axis.
- A useful command to re-scale the intensity axis to the maximum peak height in the selected range is to select **Ctrl -** å

4.1.2 How to Zoom Out to the Full Data Set

To zoom out and display the full data set, simply select the **Home** key on the keyboard.

4.1.3 How to Move the View Window Left/Right or Up/Down

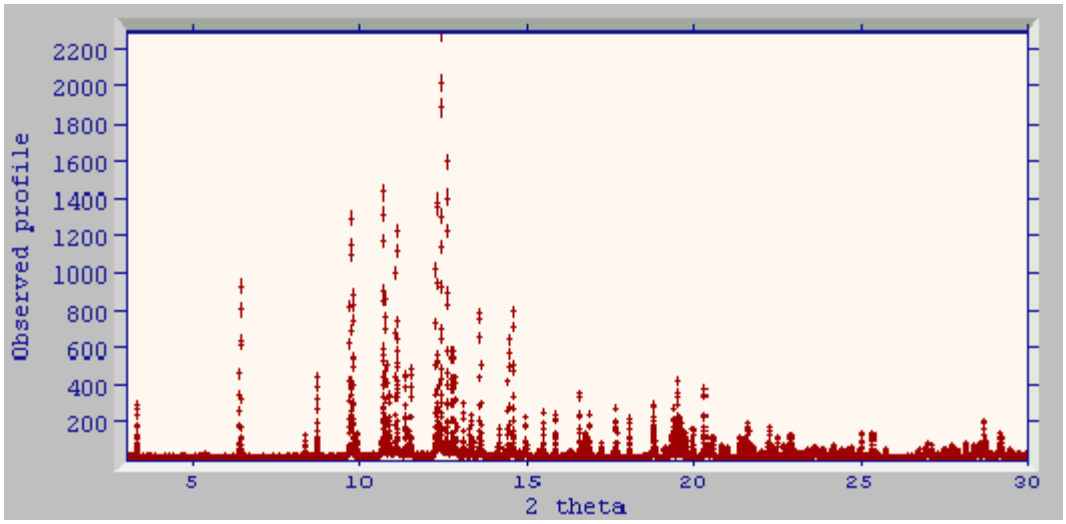
You can use the left and right cursor keys to move up left or right through the data in 2q, (the horizontal axis). Selecting the **Shift** key in conjunction with the left or right cursor keys allows the same movement, but with a smaller step size. The up and down cursor keys allow you to move the window up and down in the intensity range (the vertical axis).

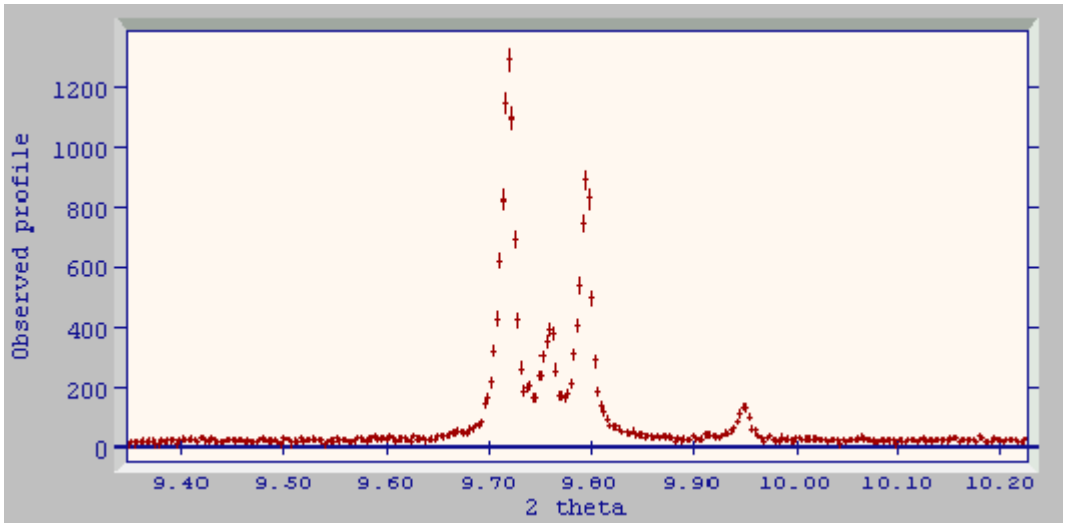
4.2 Signal-to-Noise Ratio

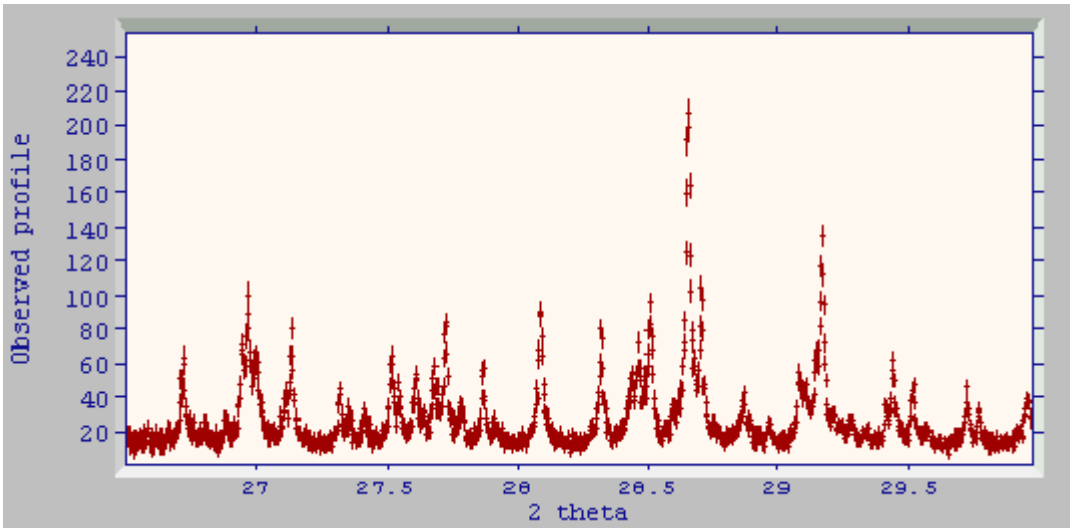
- How easy is it to distinguish the Bragg peaks from the background? Obviously, the noisier the data, the less certain we can be of obtaining a definitive crystal structure.
- The following examples should help give you some idea of what *good*, *average* and *poor* quality diffraction patterns look like:

4.2.1 Example of a Good Profile

This is synchrotron data from a nicely crystalline sample, with an incident wavelength of 0.6 Å. The background is low and the peak-to-background ratio is excellent, even at high angles. Individual esds of each point (displayed as vertical bars) are relatively small, showing that data have been collected for a sufficiently long time:

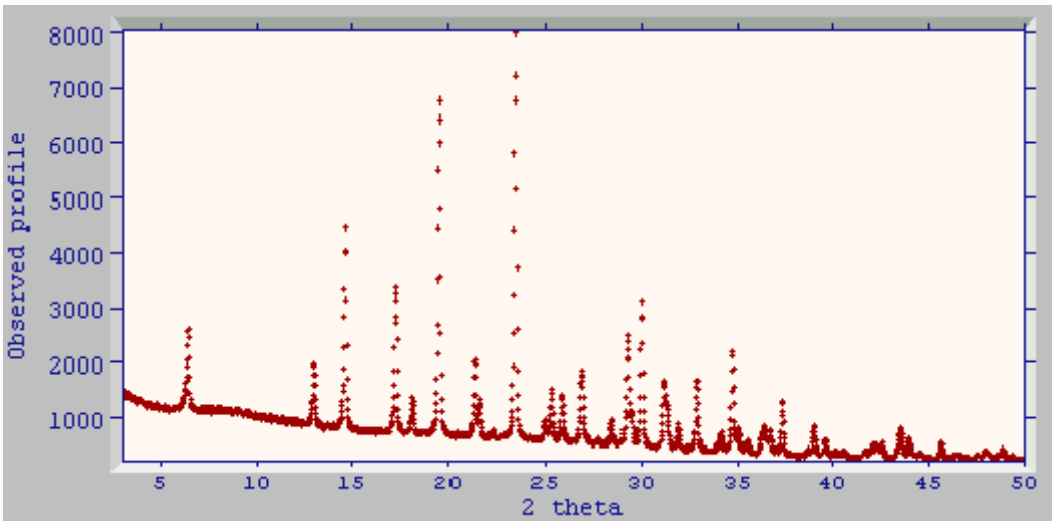

Observed profile
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
5
10
15
20
25
30
2 theta

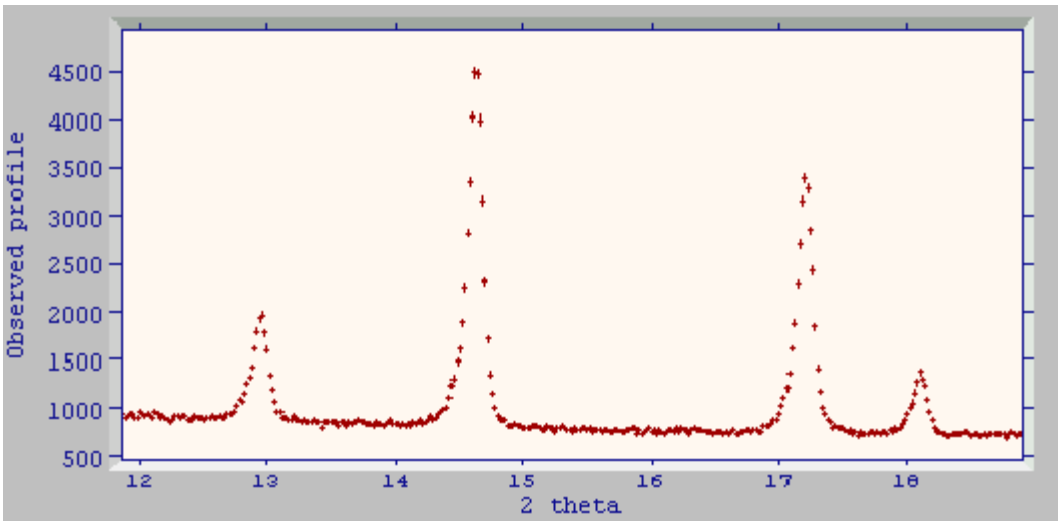

Observed profile
1200
1000
800
600
400
200
0
9.40
9.50
9.60
9.70
9.80
9.90
10.00
10.10
10.20
2 theta

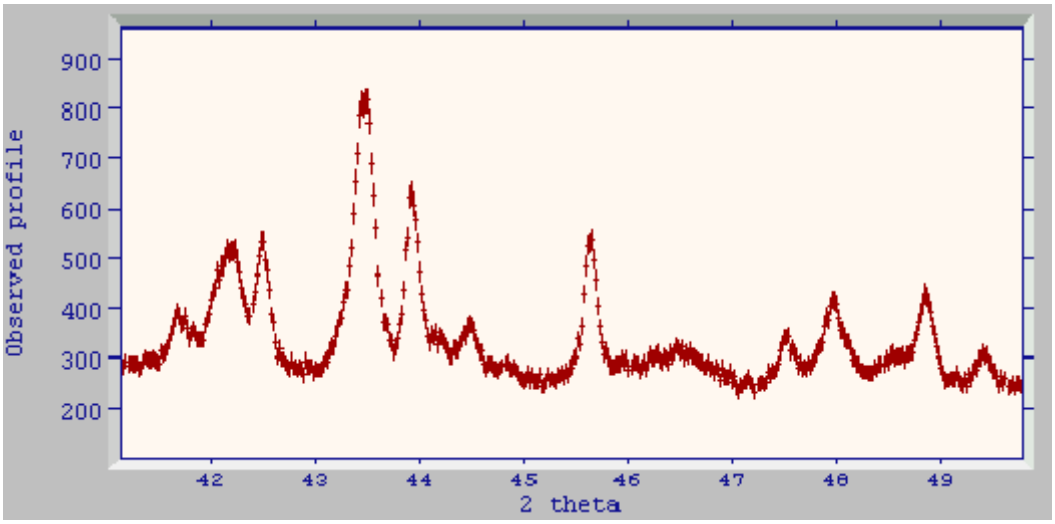

Observed profile
240
220
200
180
160
140
120
100
80
60
40
20
27
27.5
28
28.5
29
29.5
2 theta

4.2.2 Example of an Average Profile

This is laboratory data (1.7889 Å wavelength) for a nicely crystalline sample (data by permission of Dr. L. Smrcok). The background counts are higher and the profile generally noisier than the example synchrotron pattern, but the background-to-noise ratio is still reasonable. The profile is significantly worse than the synchrotron example at high angle, but peaks are still sufficiently well defined to produce useful information for structure solution:

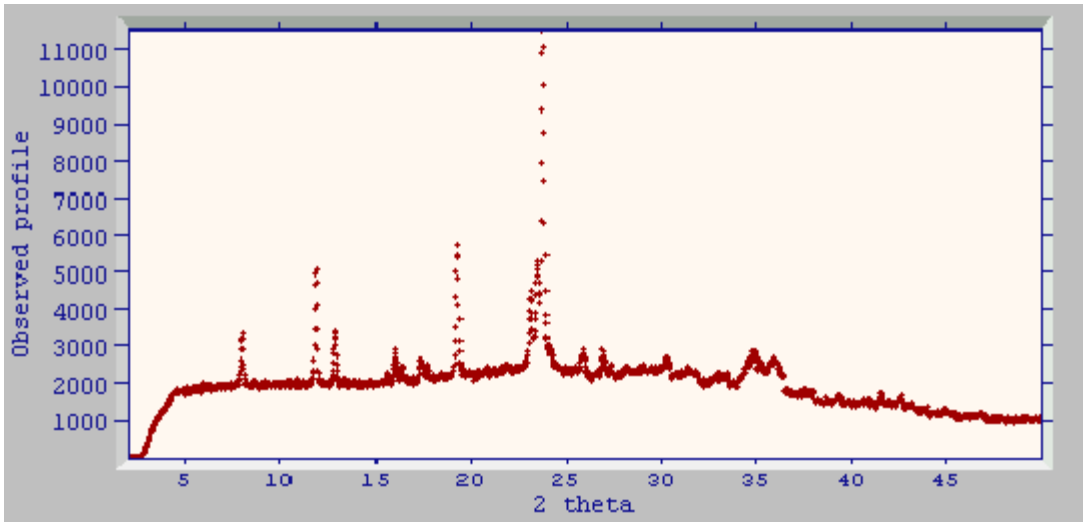


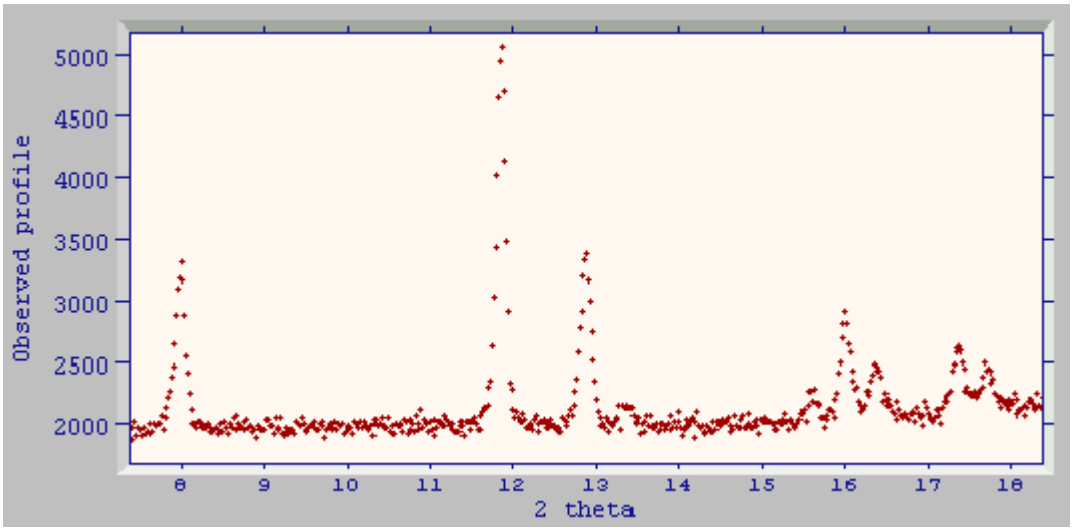


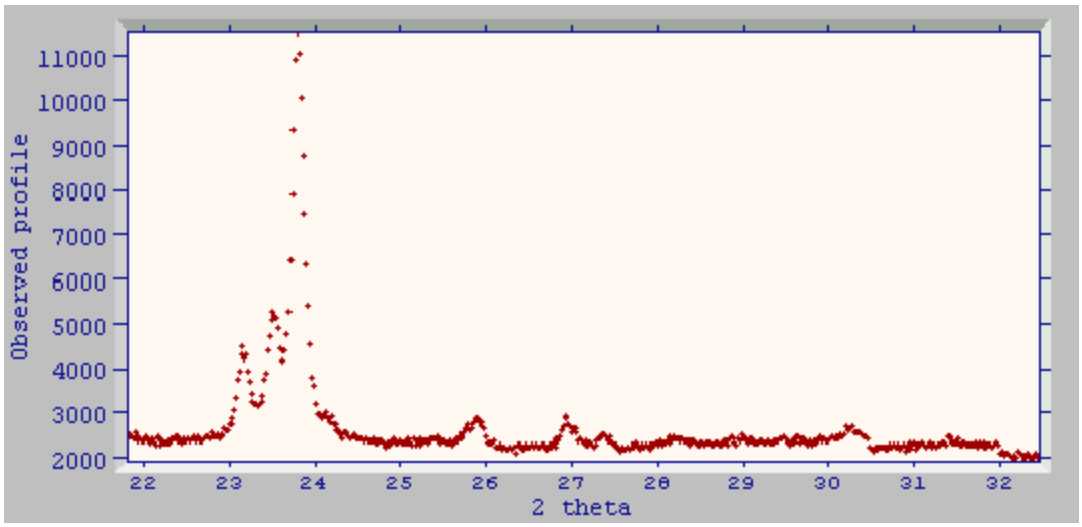


4.2.3 Example of a Poor Profile

This is laboratory data (1.54056 Å wavelength) for a rather poorly crystalline sample (data by permission of Dr. J. P. Attfield), the peaks are fairly broad and background is high, with little diffracted intensity beyond about 25^{o}:







Note: The above data were actually sufficient to solve a problem involving 7 variable torsion angles. Remember that weak peaks, provided that they are sufficiently well determined, are just as powerful a constraint on the solution as strong peaks.

4.3 Initial Assessment of Esds

- The error bars on the data points should look similar to those shown in the example profiles. If they look significantly bigger, then there could be a problem with the esds.
- If you have been given a data set and you suspect that the esds are incorrect, then you can always replace them with the square roots of the counts, or delete them from the input file and let DASH calculate them.

4.4 Initial Assessment of Background Shape

- Backgrounds may be largely flat, sloping, or rising-and-falling, as illustrated in the example

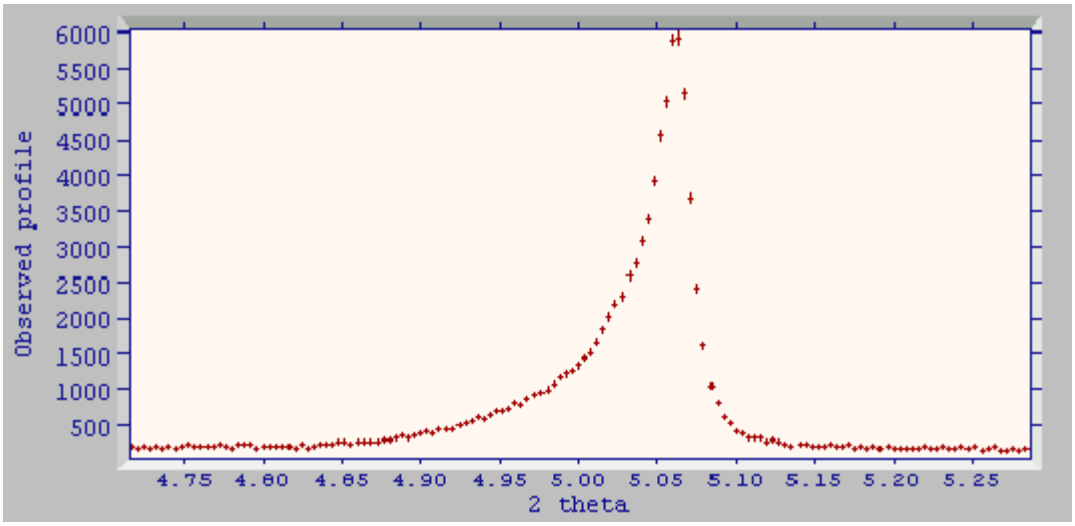
profiles.

- During data input, a Monte-Carlo background estimation routine gives you the chance to fit and remove the background. You should normally use this background removal option.
- During Pawley fitting, a 2nd order polynomial is then sufficient to represent the background. If you did not take the background subtraction option, then a higher order polynomial will be used. The more complex the background, the more terms might have to be used in this polynomial.

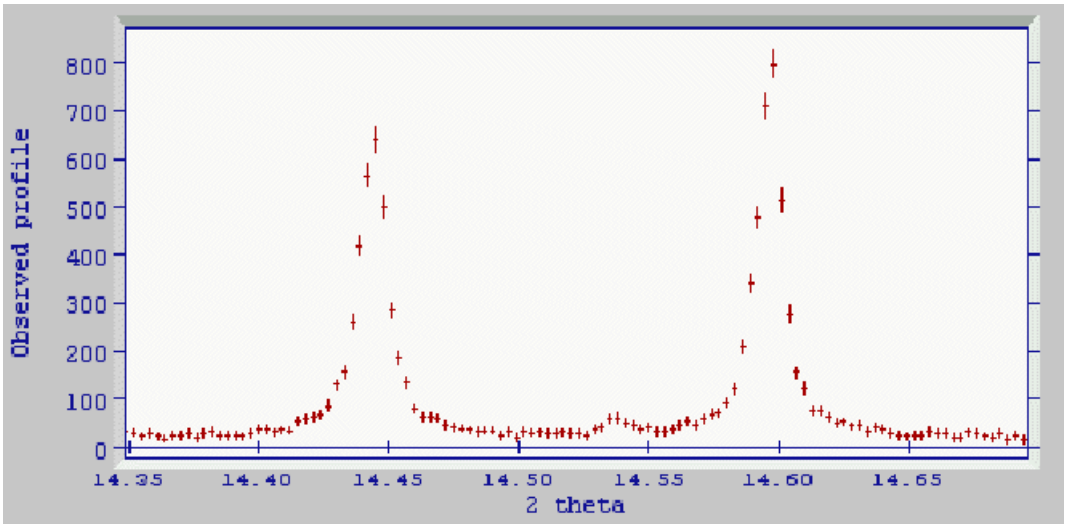
4.5 Initial Assessment of Peak Shapes

DASH is able to fit the majority of peak shapes that you will encounter in diffraction from organic compounds, including asymmetry at low angles due to axial divergence.

Asymmetry due to axial divergence at low angle:



Symmetric peaks at moderate resolution:



- When visually assessing a diffraction pattern, it is useful to remember that, at low angles, peaks appear broadened by asymmetry. At high angles, peaks begin to overlap. Thus, it is probably best to assess the overall peak sharpness from the low to mid range section of the diffraction pattern, where the probability of diffraction peaks being the result of individual Bragg reflections is much higher than at high angle.
- Sharp diffraction peaks are obviously preferable, because the sharper the peaks, the less overlap there will be between adjacent peaks in the pattern.
- The most obvious reason for broad peaks in a diffraction pattern is that the compound under study possesses intrinsically broad peaks. Frequently, recrystallisation of the sample can improve matters, but normally we are stuck with the sample *as-is* and must accept the broader peaks.
- It is always possible that peaks that appear broad are actually doublets (i.e. closely spaced pairs of peaks).
- If any of the peaks are noticeably sharper than the others, this can indicate *hkl*-dependent line broadening (i.e. some classes of reflections are sharper than others). If only relatively few reflections are affected and the broadening is not excessive, this will not preclude structure solution.

4.6 Patterns Dominated by a Few Strong Peaks

If your diffraction pattern is dominated by just a few very strong peaks, the following possibilities exist:

- The distribution of intensities may be correct. For example, this type of pattern will result if a planar molecule is lying so that the bulk of its scattering power is concentrated within a few *hkl* planes.
- Weak peaks, provided that they are sufficiently well determined, are just as powerful a constraint on the structure solution as strong peaks.
- The distribution of intensities may be indicative of preferred orientation, i.e. the crystallites in the powder sample were not randomly oriented with respect to the incident radiation, but tended to be aligned along a certain direction. Preferred orientation is not usually a big problem if transmission capillary data has been collected. Whilst, in principle, the direction and extent of preferred orientation within the sample can be determined as part of the structure solution process, in the current version of DASH only the extent of the preferred orientation can be optimised during the simulated annealing.
- In rare cases, a large peak may turn out to be an instrumental artefact e.g. a *spike* in the detector electronics. Such *rogue* points can normally be edited out by hand.

4.7 Flattened Peak Tops

If strong peaks in your diffraction pattern appear to have flattened tops, it is likely that the detector has been saturated during the data collection. If the flattening has seriously truncated the height of the peak, you will not be able to obtain an accurate intensity value for the peak during Pawley fitting.

4.8 Initial Assessment of Useful 2q Range

- A simple rule of thumb for assessing the useful data range obtained in a powder diffraction experiment is to take all the data from the lowest 2q value to the highest value at which Bragg peaks are still clearly discernible from the background.
- There is little point in including data in the Pawley refinement that is above the useful range; it will merely slow the refinement down without adding useful information. In extreme cases, it may actually hinder structure solution, as unreliable information has been introduced into the problem.
- Structure solution does not normally require as much data as structure refinement. Diffraction data up to 1.5 Å resolution are normally sufficient for a successful structure solution, though in many cases, data to 2.0 Å or even lower resolution will suffice.
- DASH will handle up to a total of around 600 refinable intensities during the Pawley fitting process.

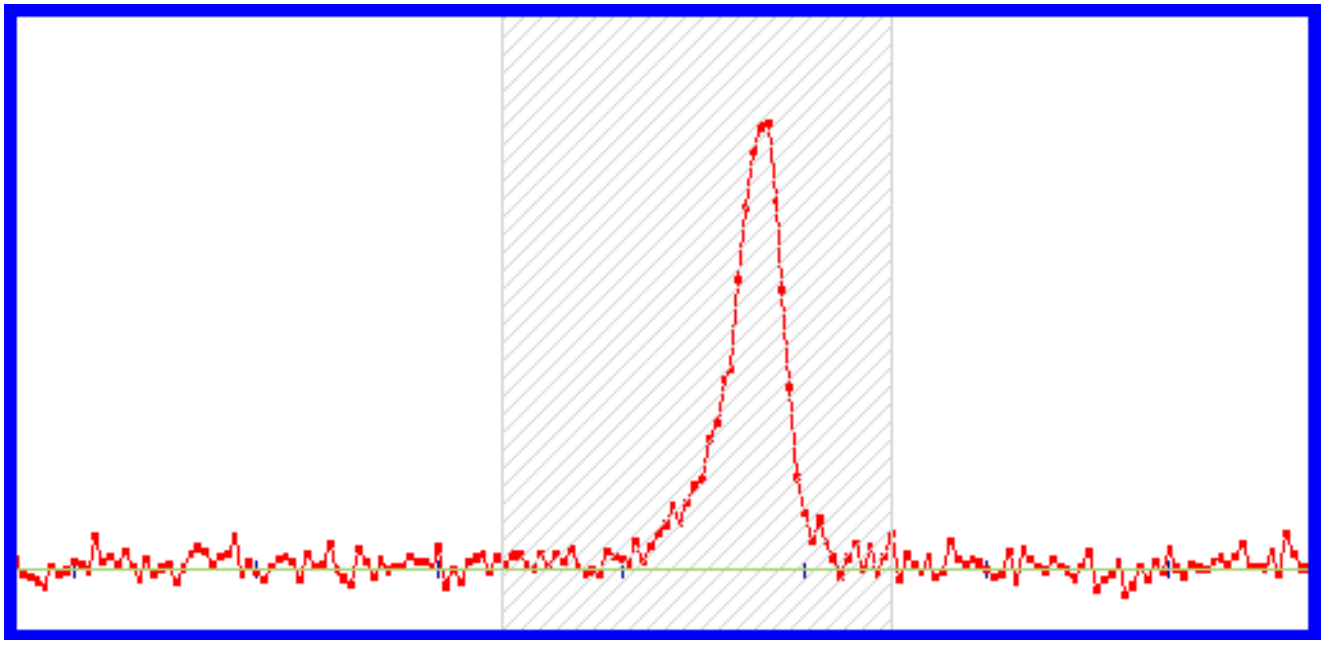
5 GENERAL HINTS ON SELECTING, FITTING AND MEASURING PEAKS

Selecting peaks in DASH is necessary for two different reasons. Firstly, it is necessary for measuring accurate positions of low-angle peaks for input to a cell indexing program. Secondly, it is necessary to fit the shapes of a number of peaks across a wide 2q range before performing a Pawley refinement. This section is applicable to both situations and gives some general advice on:

- How to use the interface to select peaks (see Section 5.1, page 47).
- The basics of peak fitting (see Section 5.2, page 47).
- Fitting multiple peaks and shoulders (see Section 5.3, page 49).
- Common problem situations (see Section 5.4, page 51).

5.1 How to use the Interface to Select Peaks

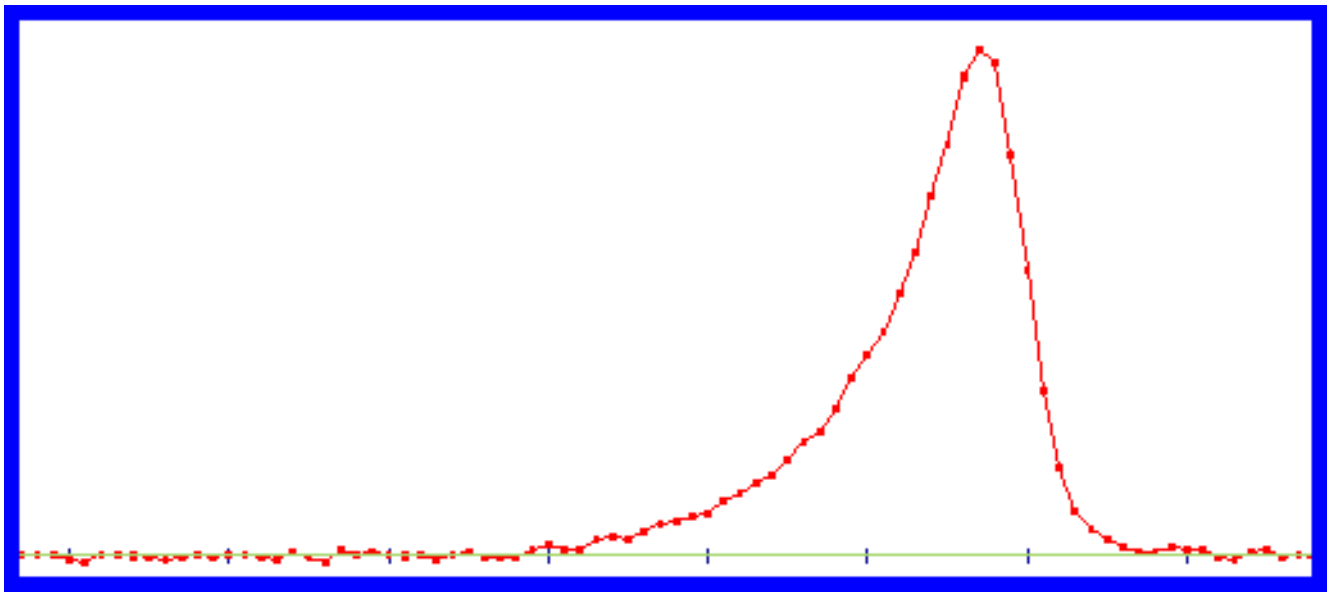
- Zoom in to the area around the peak.
- Sweep out an area using the right mouse button, in this example move to about 6.85^{o} 2q, click right and hold down as you sweep right to about 7.05^{o} before releasing the right button. The hatched area now covers the peak and enough background either side to allow an accurate estimate of the peak parameters. If you are not happy with the area that you've swept out, simply put the mouse cursor inside the hatched area and select the **Delete** key on the keyboard to remove the current selected area, then try again.



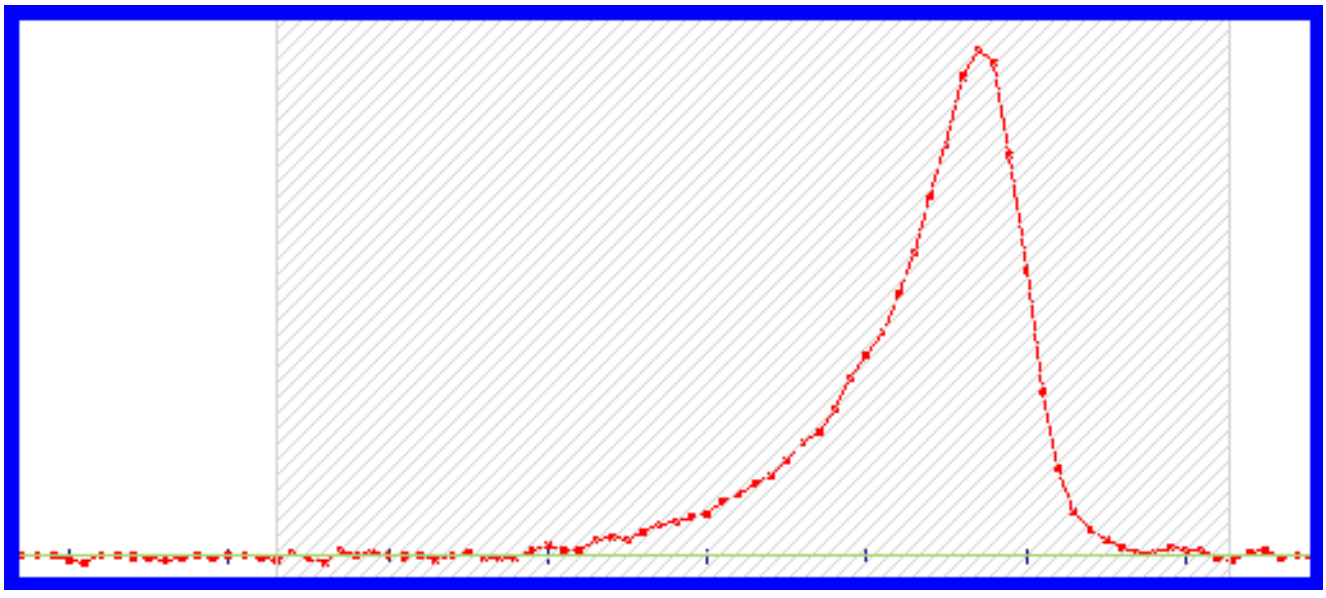
5.2 Basic Peak Fitting

- When you first read experimental data into DASH, it will be displayed in the resizable graph

window. Identify the first peak in the diffraction pattern and zoom in on it, remembering to include a little baseline either side of the peak if possible:

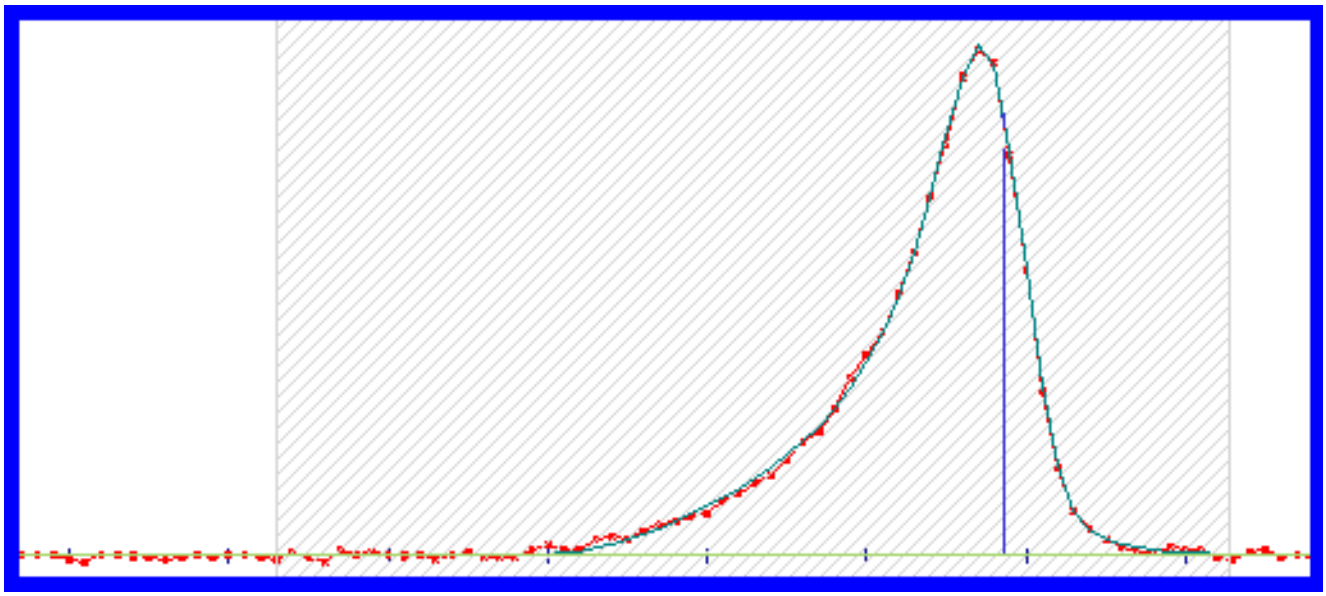


- Sweep out a selection area by clicking and holding the right mouse button, remembering to include a little baseline either side of the peak if possible:



- To fit the peak in a hatched area, either press **Return** or **Enter** with the cursor positioned inside that area, or press the following **Fit peaks** button from the toolbar:

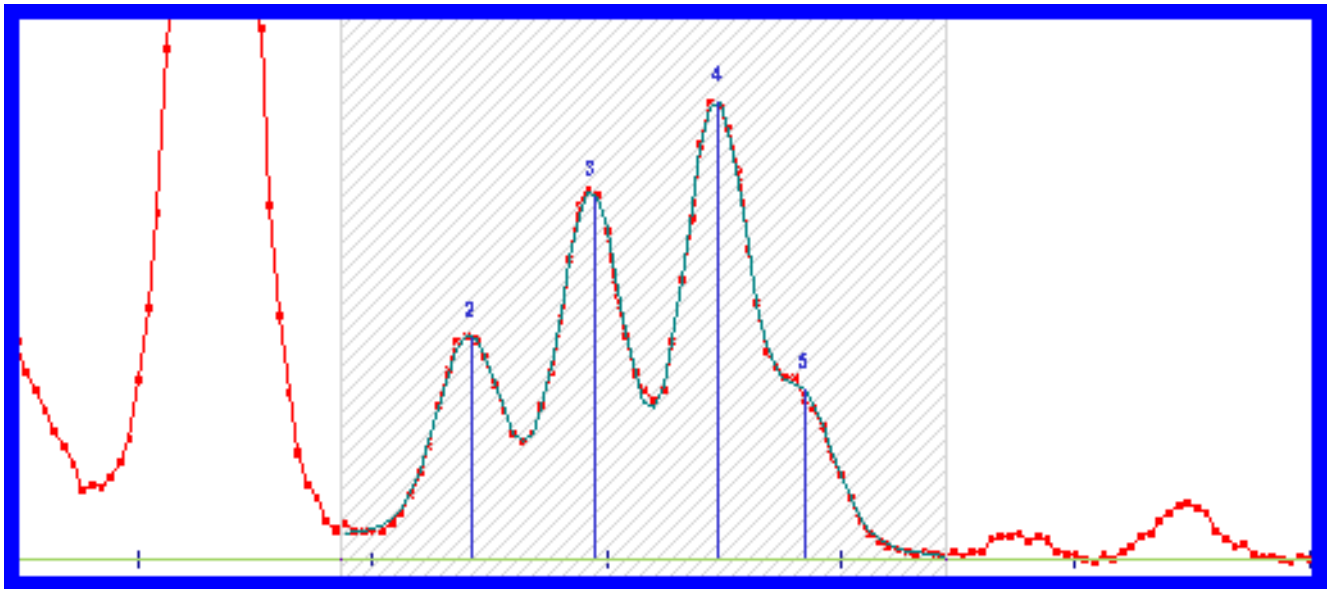
Selecting this button will fit all areas that have not yet been fitted. The peak is fitted and the fit displayed as a solid green line. The peak position is indicated by a vertical blue line:



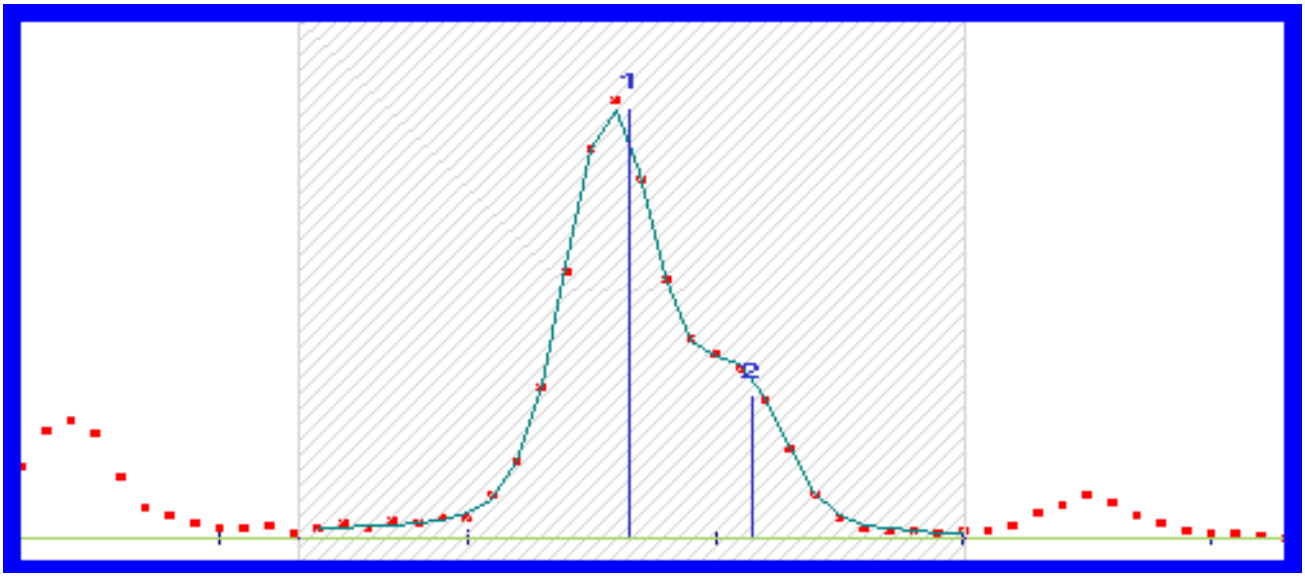
- The program will tell you if you select too small an area for peak fitting.
- If you select a larger area than is necessary for defining the baseline around a peak, no harm is done, as long as you do not stray into the next peak along. The fitting process simply takes longer as more points have to be considered in the fit.

5.3 Fitting Multiple Peaks and Shoulders

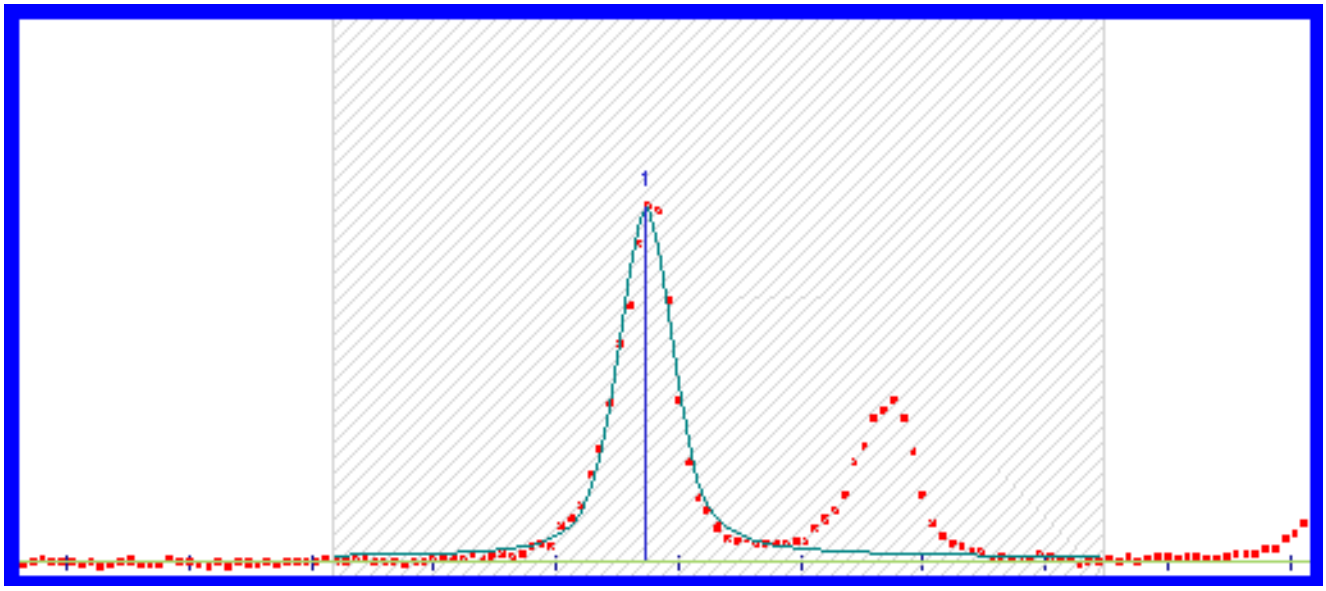
- More than one peak can be fitted at once. Select an area and give the program the position of the first peak in the region by moving the cursor over the top of the first peak and selecting **1** on the keyboard. Move on to the top of the second peak and select **2**. Alternatively, new peak positions can be added by pressing the **Insert** key. Continue until all the peaks you think are present are accounted for. Upon selecting **Enter**, all peaks are fitted and their true positions indicated:



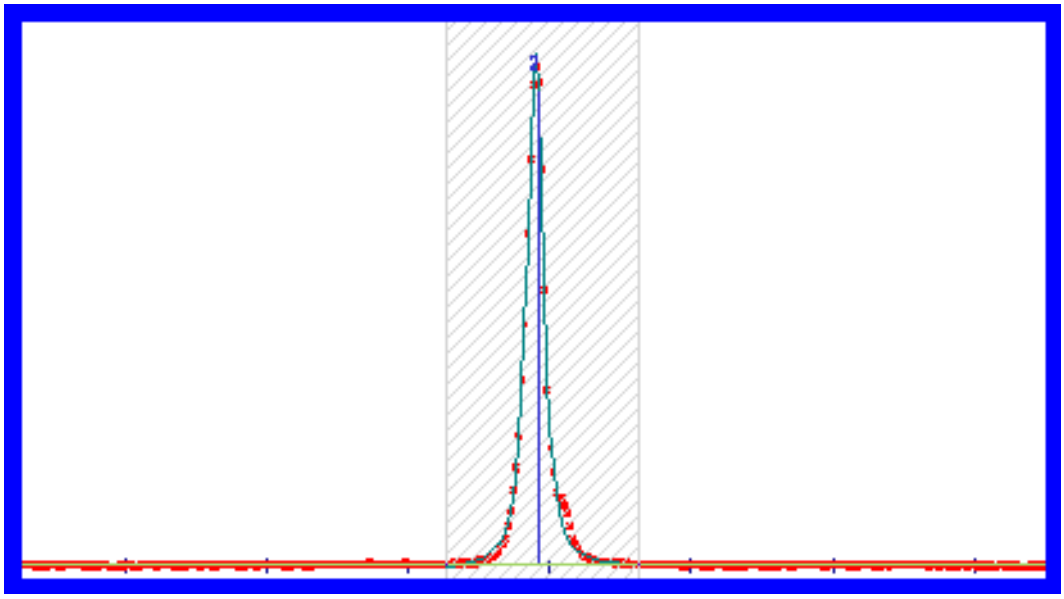
- Care must be taken to correctly fit peak shoulders due to very closely spaced Bragg reflections:



- Selecting an area containing several diffraction peaks, but fitting only one peak (i.e. the default fit), results in a poor fit. This can range from the obvious i.e. an entire independent peak is missed out:



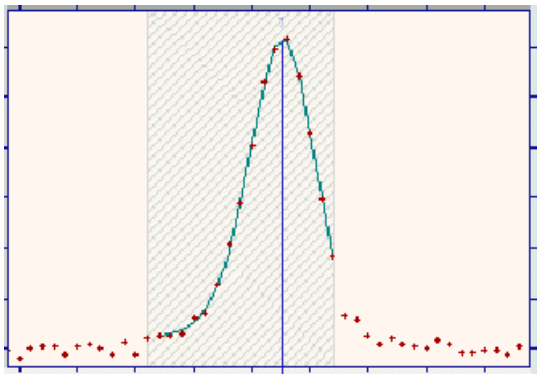
to the more subtle i.e. a shoulder on a peak is missed:



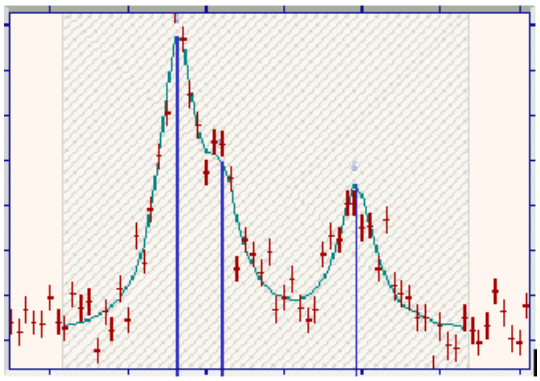
5.4 Common Peak Fitting Problems

- There are a few circumstances in which you might obtain a poor fit to the data:
  - If you are trying to fit a very weak peak with large esds.
  - If you fail to select an appropriate region in which the fit will be performed.
  - If you do not enter enough peaks to properly describe the data in the selected area.
  - If you enter far too many peaks in the selected region.

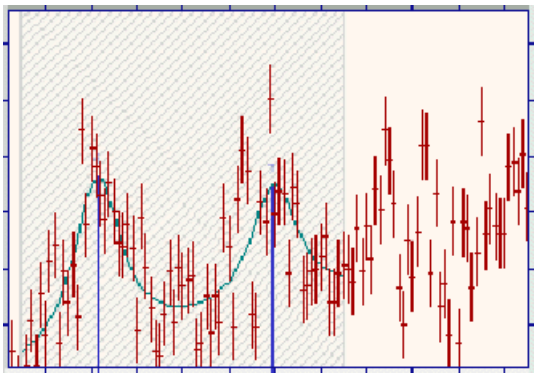
- In such circumstances, the peak fitting algorithm may converge to a local rather than a global minimum. This is usually obvious, as the calculated peaks fail to match the data. It is trivial to delete a poorly fitted region and try again.
- By way of reassurance, here are some extreme examples where the peak fitting algorithm has still produced a useful result:



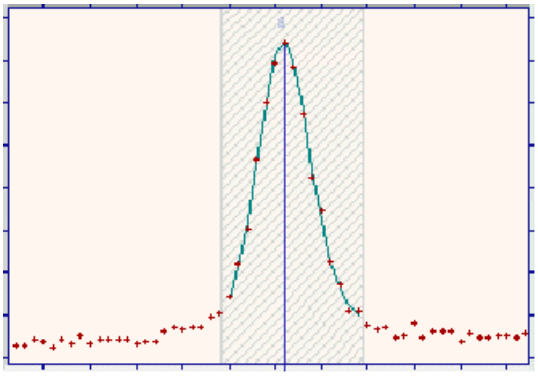
Selected range too short on the right



Three closely spaced weak peaks



Two very weak peaks with large esds



Selected range too short on both sides

6 INDEXING

6.1 Overview of Indexing

- Correct indexing of the powder pattern (i.e. determination of the unit cell dimensions) is crucial: you cannot solve the structure if the pattern is not indexed properly. DASH can help you with indexing by allowing you to determine peak positions with great accuracy. However, DASH does not do the actual indexing itself. For this, you must use one of the many, freely available cell-indexing programs. DASH does provide an interface for DICVOL (see Section 6.2.6, page 58), which is convenient for many users.
- However, it is strongly recommended that one should use at least two indexing programs. The freely available CRYSFIRE suite of Shirley provides a rudimentary interface to most of the popular indexing programs, e.g. DICVOL and ITO and is currently available for download from *http://www.ccp14.ac.uk*.

The steps involved in indexing are:

- Selecting the first 20 or so low-angle peaks and measuring their positions for input to the indexing program. Of course, only the positions of the lines are important for indexing, not their intensities. It therefore follows that weak peaks carry just as much weight as strong ones in the indexing process (see Section 6.2, page 55).
- Indexing the pattern to find a plausible set of cell dimensions (see Section 6.3, page 59).
- Checking for possible cells of higher symmetry (see Section 6.4, page 61).
- Checking the cell in DASH by comparing observed and calculated peak positions (see Section 6.5, page 61).

6.2 Selecting Peaks for Indexing

6.2.1 Overview of Peak Selection for Indexing

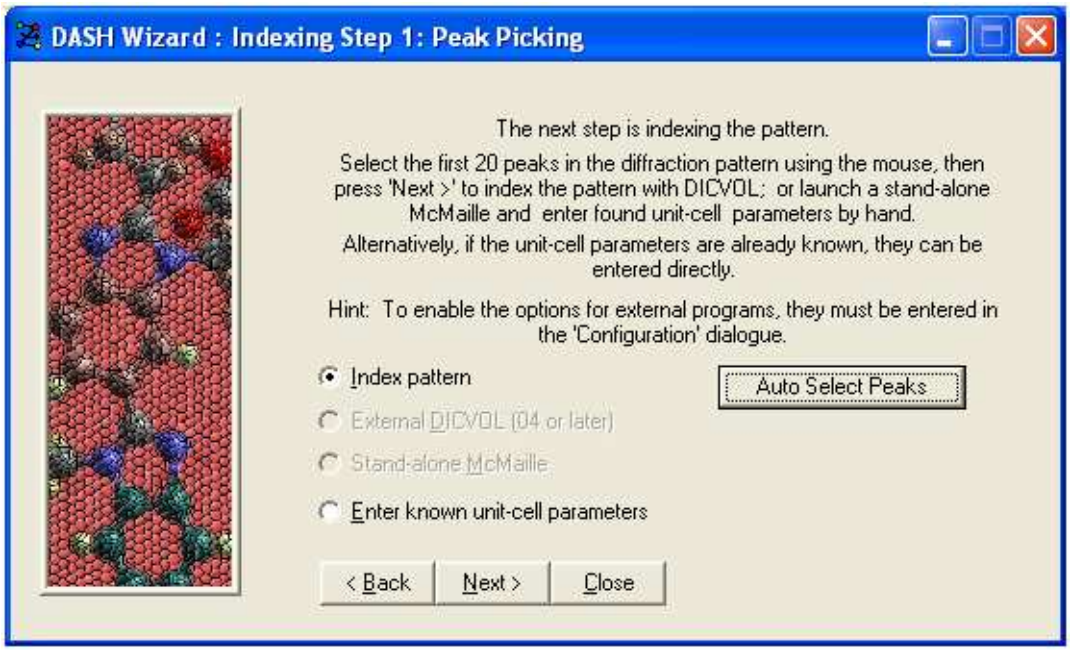
- You should select the lowest-angle peaks available, regardless of their intensity, to ensure that the indexing program has a chance to find the correct cell.
- In general, you should pick the first 20 peaks or so (including shoulders), trying not to miss any.
- As long as a peak is clearly present, you should pick it, even if it is weak; but if you are not sure the peak is significantly above the background, you can leave it. On balance, if you are at all uncertain about a peak or shoulder, it is probably better to include it in the first instance. You can easily edit it out of the peak list later if the pattern fails to index.
- As successive peaks are selected, DASH will refine the peak shape parameters.
- Submit these peaks to a cell-indexing program such as DICVOL or McMaille in order to obtain a preliminary unit cell and crystal system.

6.2.2 How to Use the Interface to Select Peaks for Indexing

- Zoom in to well resolved single peaks, working from lowest 2q upwards.
- Pick the peak using the right mouse button as described in Section 5.1, page 47.
- Continue picking peaks (the peak count appears beside the blue peak position line).
- Finish peak picking when you have between 20-25 peaks.

6.2.3 Auto peak picking for indexing

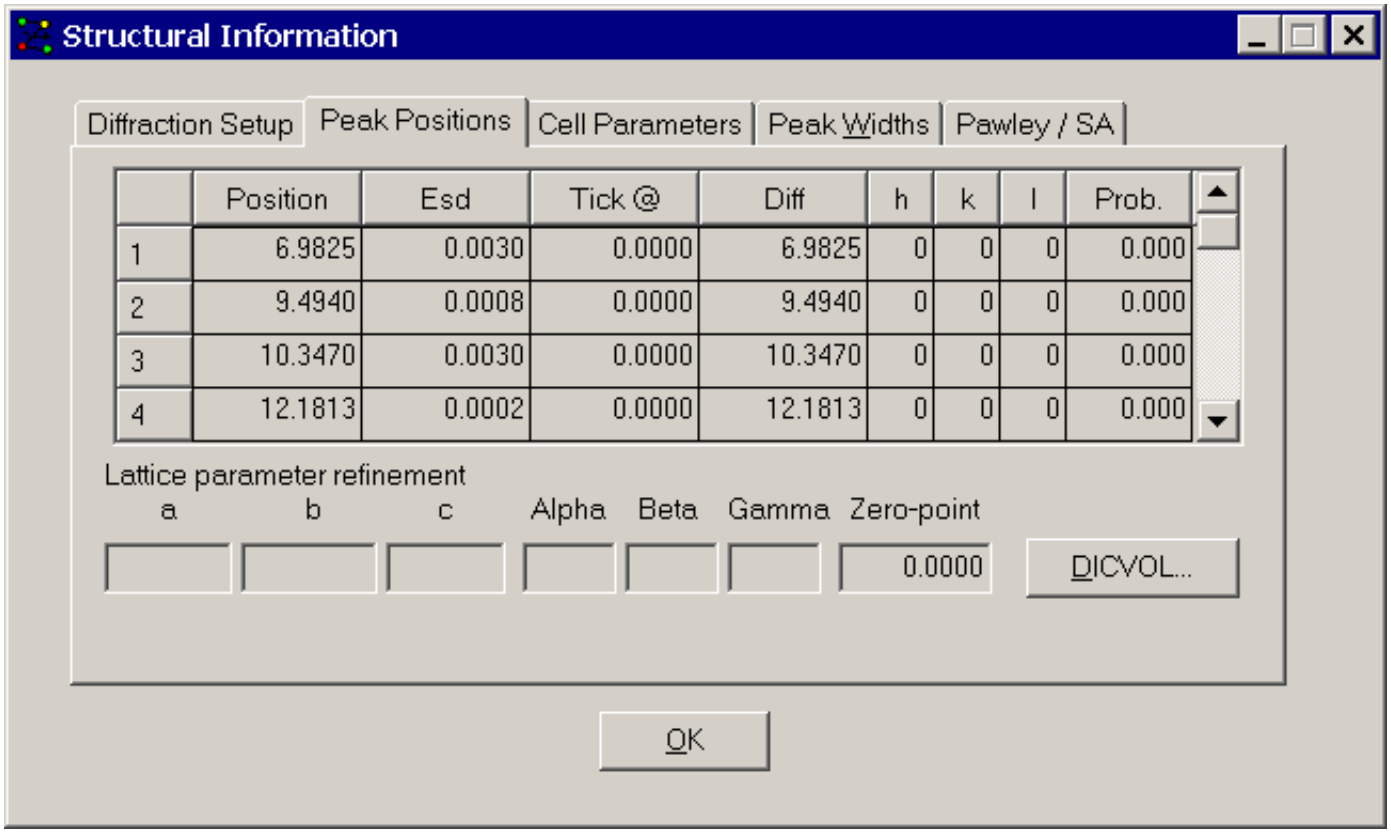
- DASH contains a heuristic algorithm for automatic peak picking. The algorithm first smoothes the powder pattern by window integration in a fixed 2q range of ±0.012^{o} around each point in the profile. The window width has been determined by experimentation with several sample patterns; it seems that this width offers a good trade-off between effective smoothing for patterns collected at narrow 2q step sizes and masking of peaks in patterns collected with broader step sizes.
- Next, the pattern is scanned for regions where the intensity is greater than 8 times the residual background. Auto-peak selection is habitually applied after background subtraction in DASH, and will not work well on patterns where background has been left in place. In regions where intensity is greater than 8 times the residual background, high points in the smoothed intensity are detected and peaks are assigned to these locations. In cases where multiple peaks are found that are close in 2q, peaks are merged.
- Using Auto Peak is easy. In the Indexing Wizard, the user reaches the following window: The user can click on the **Auto Select Peaks** button and DASH will attempt to fit ranges automatically. The feature is designed not to be a ‘black box’; after completion, the user is encouraged to review the assignments made as the heuristics in the algorithm are not perfect.



- The peaks fitted can be modified and/or deleted after automatic peak fitting by hand. Further, the user can add in more peaks manually if they so wish.
- Automatic peak picking is not perfect, and as such should not be treated as a black box; often the peaks picked are sufficient for indexing purposes, but still the user should review the results carefully after use. The following caveats exist:
  - Shoulders on peaks are rarely detected by the current algorithm
  - Occasionally, a single actual peak can be assigned 2 or more peak positions, particularly when said peak is broad (e.g. a t low 2q)
  - Unfitted background renders the algorithm useless
  - We occasionally miss weaker peaks
  - Some strong pairs of peaks are sometimes misinterpreted as a single peak. This case is a bug in the code which remains unresolved at time of writing.
  - Only the first 20 peaks are fitted: this means the results for the 18^{th} - 20^{th} peaks can appear strange when the 20^{th} peak overlaps with other higher angle peaks (it can appear that the algorithm has missed the higher angle peaks as they will lie in the fitting range, but in fact, the algorithm has decided to ignore the higher angle peaks.)

6.2.4 How to View Peak Positions

- Switch to viewing peak positions by selecting **Peak Positions** from the **View** menu:



6.2.5 How to Cut and Paste Peak Positions to External Programs

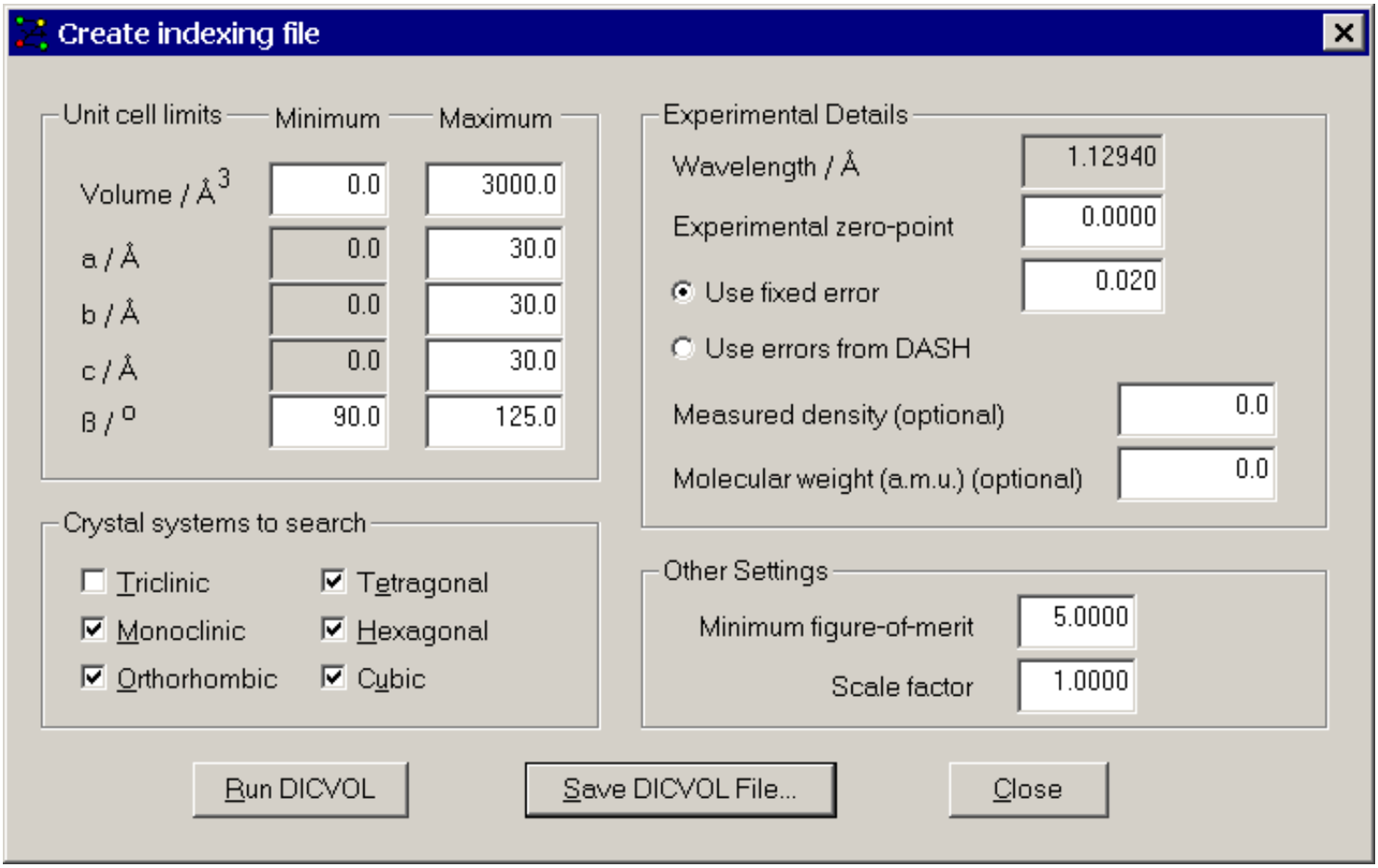
Note that if you want to copy the set of peak positions into the notepad for feeding to other programs, you can easily get the peak positions out of DASH and into a file as follows:

- Select **View** from the **Peak Positions** menu and then click on the word **Position** at the top of the peak position column. This selects the entire column.
- Use **Ctrl-C** to copy the entire column to the clipboard.
- Once inside an appropriate editor such as Notepad or Wordpad, use **Ctrl-V** to paste the column into a file.

6.2.6 How to create a DICVOL91 input file

- Switch to viewing peak positions by selecting **Peak Positions** from the **View** menu.
- Create an input file for DICVOL using the **DICVOL...** button (see below).
- Fill in the information required on maximum values allowed for cell axes and volume.
- Click **Save DICVOL File...** button. Give a file name e.g. *hctpeak21.dat.*
- Run the DICVOL program with this file as input.

- The menu for creating a DICVOL91 file is shown here. In the example below, values have been entered such that: minimum cell volume = 0 Å^3, maximum cell volume = 3000 Å^3. The maximum cell axial length is set to 30 Å, and the maximum monoclinic cell angle is 125^o. All crystal systems except triclinic will be searched, and the wavelength has been set as 1.1294 Å. As it is synchrotron data, DASH has set the peak position error to 0.02 by default (0.03 for laboratory data). The other values have been left at their default setting, and will not affect the DICVOL result.



Alternatively, indexing may be performed using the Wizard (see Section 2.10.1, page 22).

6.3 Running the Indexing Program

- DASH provides an automated interface to DICVOL91, just click **Run DICVOL**.
- DASH also provides an interface to DICVOL04(and later) and McMaille. The interfaces to these programs can be accessed through the *Peak Picking* wizard window.(see Section 2.10.1, page 22)
- There is no automated interface between DASH and indexing programs other than DICVOL91, DICVOL04 and McMaille, i.e. it is necessary to set up the input files for the programs by hand. However, this task is facilitated by copying the peaks from the DASH Peak Positions listing into the notepad (see Section 6.2.5, page 58).

- Each indexing program has its own strengths and weaknesses. We have found DICVOL, TREOR and ITO to be useful when indexing organic crystal structures, but this is not to say that other programs will not be equally successful.
- Frequently, one program will successfully index a pattern where another has failed. It is therefore always worth trying at least two indexing programs on each problem.
- Before running an indexing program, it is useful to get some idea of the size of cell you might expect, given the molecular formula. For example, a molecule comprising 20 non-hydrogen and 25 hydrogen atoms will occupy about 450 $Å^3$ (allowing 20 $Å^3$ for each of the non-H atoms and 2 $Å^3$ for each of the H atoms). Therefore, a good starting point would be to search for cells of up to ~2000 $Å^3$ in volume, as a cell of this size will accommodate four molecules i.e. Z=4, a likely number when dealing with organic structures. You can always increase this size limit later if you do not get any reasonable cells from the initial runs.
- The majority of indexing programs were designed for use with relatively small unit cells. Advances in structure solution mean that people are tackling larger and larger crystal structures, thus stretching indexing programs to their limits and beyond. For example, it is not unheard of for TREOR to suggest that a unit cell is too large and that the data should be checked, even when the cell is correct. A useful trick here is to simply divide all the line positions by two and try again. The cell that results will be 8 times too small, but you simply double the axial lengths to recover the correct cell. DASH offers this option through the use of a scale factor.
- By default, DASH creates a DICVOL input file in which the axis lengths is limited to 30 Å or less. Since organic structures may contain a cell axis of length >30, you may need to alter this default if initial indexing fails. Similarly, you may need to increase the volume limit beyond 3000 Å if you are dealing with a large structure or a centred cell.
- The majority of organic crystal structures crystallise in monoclinic, orthorhombic and triclinic space groups and you should check these symmetries first. Within DICVOL and McMaille, the crystal symmetries are searched in order, from highest to lowest symmetry. As the symmetry falls, the cell searches take longer to execute. It can take quite a while (possibly 2-3 hours, even on a fast processor) to find triclinic cells using DICVOL or McMaille. This is the main reason why triclinic cells are not searched by default, so don't forget to try triclinic if your initial indexing attempts fail.
- Some indexing programs (e.g. TREOR) will report if they have located a non-primitive unit cell whereas other simply report the equivalent primitive cell.
- Like most indexing programs, DICVOL gives two figures of merit, M(#lines) and F(#lines), for identifying the best solution. For synchrotron data, M(20) values of 50 or more and F(20) values of 100 or more are encouraging. Values for laboratory data will be generally lower – an M(20) of, say, 20 or more might be considered reasonable and worthy of pursuit. McMaille reports a number of figures of merit for its solutions and even suggests cells that it judges to be worth investigation.
- Multiple solutions (i.e. several possible unit cells) are a common occurrence, especially when

the input data are not especially good. However, even when the data are good, a program may report two or more unit cells that apparently match the data. In such circumstances, the solutions should be closely examined. If all the cells have almost identical cell volumes, then they are likely to be alternative settings of the same cell, and any one of them could be used. This can be checked by cell reduction. If, however, the cells are markedly different in volume, then they are likely to be unrelated and each one needs to be examined more closely.

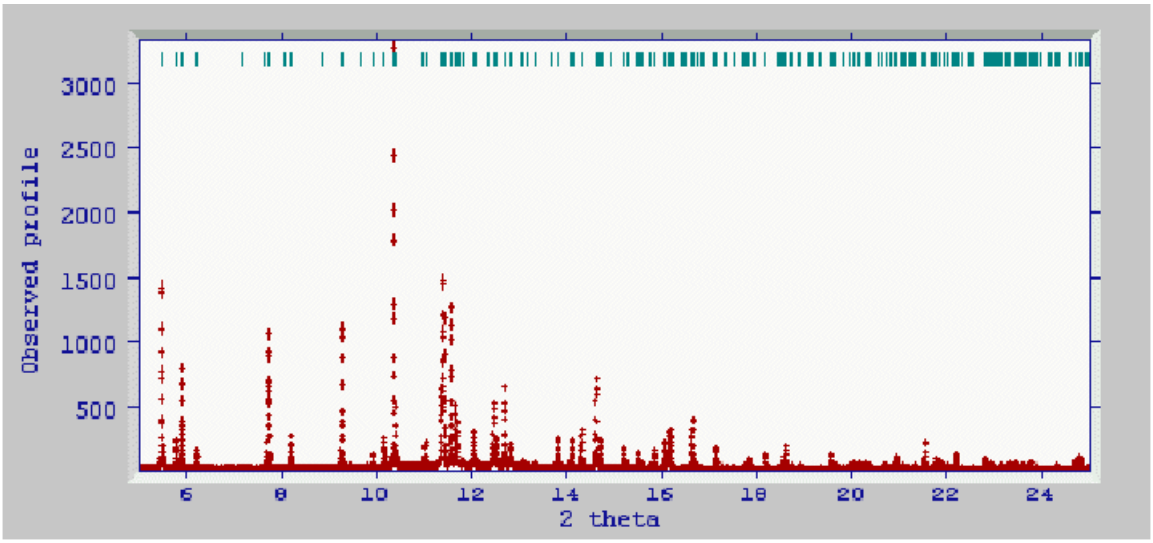
- If you find several cells, all with good figures of merit, the correct cell is likely to be the one of highest symmetry.
- A large number of low figure-of-merit solutions is normally a bad sign – it indicates that the input positions are sufficiently vague that a number of cells match to within experimental error.
- If you have trouble finding a cell, it is sometimes worth deleting the last 3 or 4 peaks from the input, e.g. try with the first 16 rather than the first 20 peaks. Or try deleting very weak peaks or dubious shoulders. It is important to realise that DICVOL is more tolerant of missing lines than it is of spurious lines. Most importantly, try another program.

6.4 Searching for Cells of Higher Symmetry

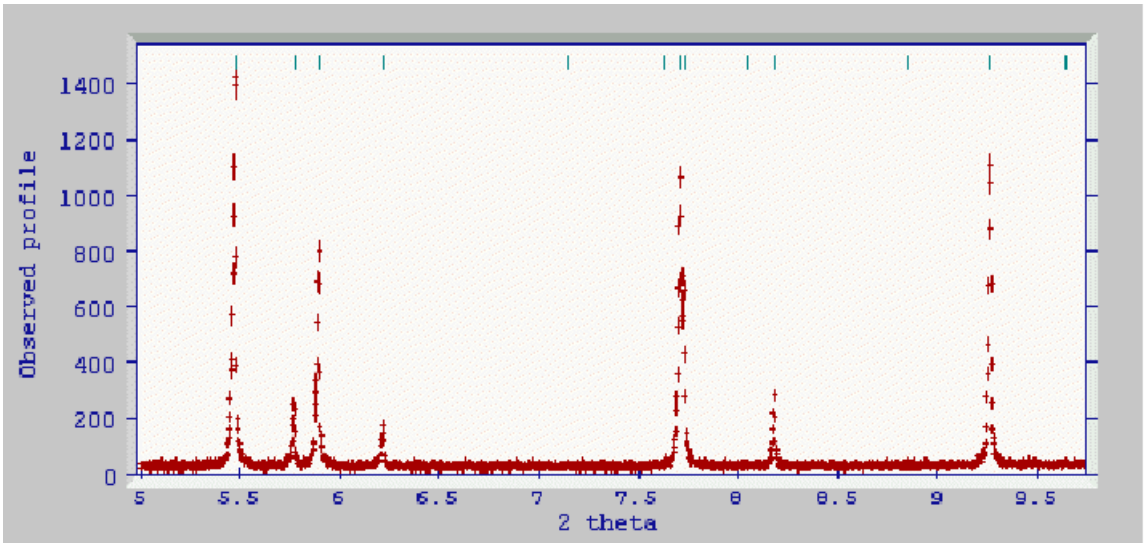
- Once a plausible cell has been obtained from an indexing program, it is worth performing cell reduction, using a program such as TRACER, to check whether it corresponds to a cell of higher symmetry.
- Searching for cells of higher symmetry is particularly important when the cell from the indexing program is triclinic. This is especially true when the indexing program lists a lot more calculated than observed peaks, since this suggests systematic absences.
- Cell reduction is also useful for identifying equivalent solutions, i.e. cells from the indexing program that appear to be different but actually correspond to the same reduced cell.

6.5 Checking the Cell in DASH

- Once a pattern has been indexed and a preliminary cell identified, you can return to DASH and input the profile and the cell. You will need to specify a space group: start with the space group of the crystal system that has no systematic absence (e.g. *P*2 for monoclinic). The pattern is displayed with *tick marks* indicating the reflection positions predicted from the input cell and space group:


Observed profile
3000
2500
2000
1500
1000
500
6
8
10
12
14
16
18
20
22
24
2 theta

- The first thing to do is to check that the tick marks actually correspond to the pattern, i.e. that the unit cell is correct. The correspondence shown below is very good, indicating that the cell is probably correct. The *excess* tick marks are probably systematic absences, indicating that the correct space group has a higher symmetry than the one currently being assumed.



7 SPACE GROUP DETERMINATION

Once the pattern has been indexed, you have a putative cell and crystal system. The next step is to determine the space group.

7.1 Probabilistic Approach to Space Group Determination

- DASH provides an interface to *Extinction Symbol*, a program that identifies the most probable space groups for a set of reflections and their intensities (see Appendix H: References, page 189). In order to furnish *Extinction Symbol* with the required information a Pawley fit to the diffraction data must be obtained, in the most general extinction group of the crystal system under consideration. For more information about the *Extinction Symbol* program please look at the product manual which includes a reference to the published research paper.
- Once the crystal system and unit cell parameters have been entered, click on **Space Group** in the *DASH Wizard: Unit Cell Parameters* window. DASH automatically sets the space group to the most general for the crystal system chosen.
- Proceed to the Pawley Refinement window (see Section 8.2, page 69). Select 6-10 individual peaks distributed over the whole 2q range of the pattern. Once DASH has stable values for the unit cell and peak shape parameters, the *DASH Wizard : Pawley Refinement Status* window will automatically pop-up.
- Obtain a good Pawley fit to the data by refining the background, unit cell and zero-point parameters. When satisfied with the fit, launch the space group determination program by pressing the **Run>** button.
- The console window for *Extinction Symbol* appears. Once the program has finished running the results of the extinction symbol determination they can be viewed by pressing **Enter** on the keyboard. The most probable extinction symbols, along with their probabilities, are displayed in descending order in the right hand column.
- When the results window is finished with, close the window. In the Pawley Refinement window click **<Back**. The files that have been generated during space group determination, including the table of results can be removed at this point by clicking **Yes** in the **Confirm** dialogue box. The files will not be deleted if **No** is chosen.
- The space group associated with the most probable extinction symbol can now be selected from the *Space Group* drop down menu. Occasionally there will be a choice of space groups for the extinction symbol returned.

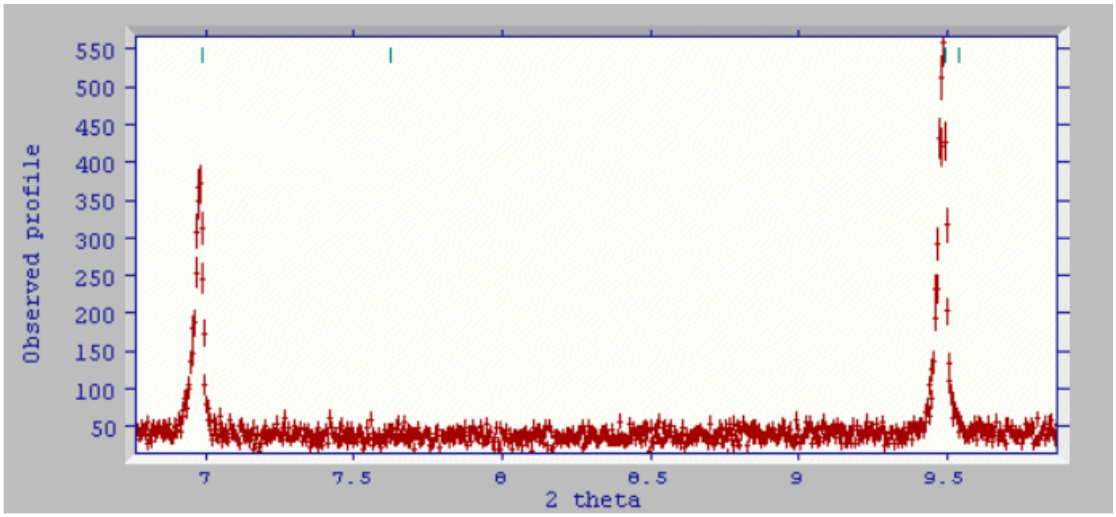
- For guidance, a table listing the extinction symbols with their associated space groups is available (see Appendix E: Extinction Symbols and their Space Groups, page 176). It may be also be useful to refer to the table of most probable space groups when deciding which space group to try first (see Appendix D: Frequency of Occurrence of Space Groups, page 159).
- Check the agreement between the calculated Bragg reflections of the chosen space group as shown by the tick marks and the peak positions of the experimental pattern. Please note that the purpose of *Extinction Symbol* is to provide guidance in identifying the space group; it is not a substitute for good judgement.
- Perform a Pawley Refinement in the chosen space group, as normal.

7.2 Identifying Systematic Absences with DASH

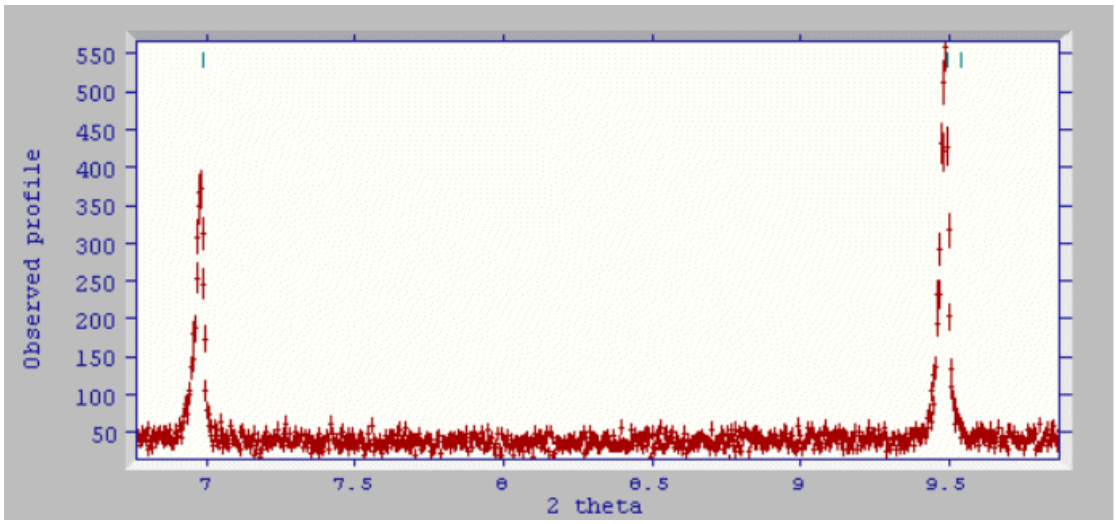
- Systematic absences can be identified in DASH by comparing, for different possible space groups, the observed peak positions with those calculated from the cell and postulated space group. These are represented by tick marks at the top of the profile display.
- Even if you already have clues about the space group, it is probably best to start by selecting the primitive space group of the appropriate crystal system with no systematic absences (e.g. *P*2 for monoclinic). Look to see if the gaps in the pattern match the gaps in the tick marks.
- Zooming the picture helps a lot when assessing whether a peak is matched by a tick mark.
- Do not worry about tick marks that are not matched by peaks in the profile: they could be systematic absences or weak peaks. However, peaks with no corresponding ticks are a warning. If you do see a peak in the profile with no matching tick mark, the cell is probably wrong, though impurities or instrument spikes should not be ruled out.
- Having examined the space group with no absences, you can now try space groups of higher symmetry to account for any systematic absences in the pattern. Browse through the space groups comparing calculated and observed peak positions.
- A useful technique is to cycle through space groups with just one cause of systematic absence (e.g. *A*2, *B*2, *C*2, *I*2, etc.) since this may enable you to eliminate a complete set (e.g. all C-centred space groups) quickly.
- It is necessary to look separately at different settings of the same space group, e.g. *P*21/*c*, *P*21/*n* and *P*21/*a*, since the cell from the indexing program could correspond to a non-standard setting.
- Although you are looking mainly for peaks in the observed pattern that do not have matching tick-marks (since this virtually eliminates that space group), do remember to look for the opposite discrepancy. If there are a lot of calculated peaks that are not observed, you may be looking for a space group with more systematic absences.
- Another way of identifying systematic absences is to perform a Pawley fit in the space group with no systematic absences. At the end of the Pawley fitting procedure, the output file *polyp.hkl* can be examined for systematic absences and the space group deduced from these absences.

7.3 Identifying Systematic Absences: an Example

- The tick marks in the following figure correspond to the space group *P*2:

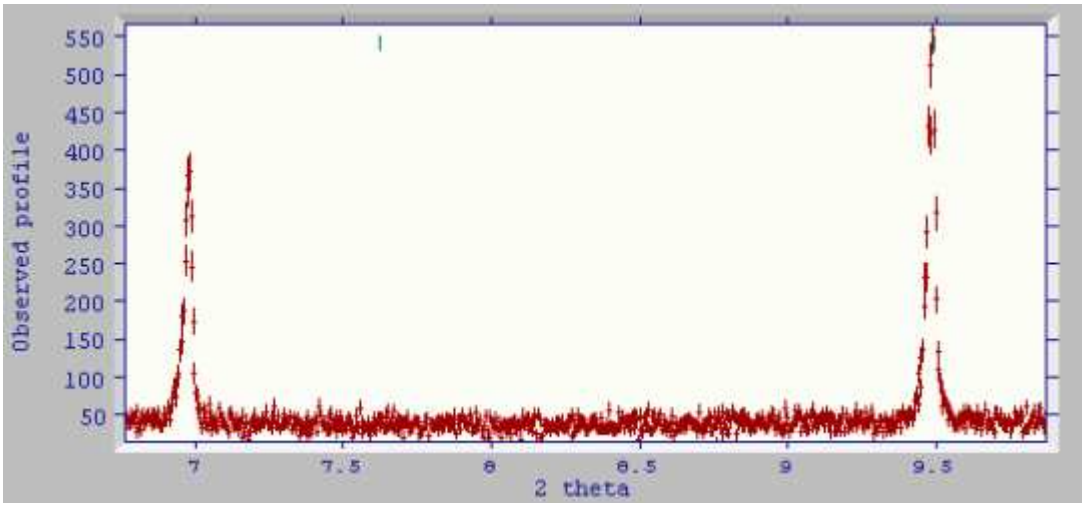


- The peak around 7.6° 2q appears to be absent. If the user changes the space group to *P* 1 2_1 1 (a likely choice) the graph is updated with a new set of tick marks:



- The predicted peak at 7.6° is an absence in *P* 1 2_1 1; other predicted peaks are consistent with the observed data and so the fit can be performed in *P* 1 2_1 1.

- If you pick the wrong space group, it is often obvious. The following display results if the space group is changed to *P* 1 *c* 1. This choice of space group is clearly wrong, since the strong peak at about 7.0° would need to be systematically absent:



7.4 Space Group Ambiguities

- If it is difficult to decide which of two space groups is a better match to the pattern, you can try toggling between them several times to identify subtle differences between the calculated peak positions. For example, you might find a point at which one of the space groups has two tick marks and the other has only one. If the peak underneath is unusually broad, this indicates that the former space group is slightly more likely.
- Inevitably, there will be many cases where the space group cannot be determined unambiguously. In this situation, there is little choice other than to try each possibility in turn, starting with the space group that has the highest statistical probability of occurrence (see D.1 Space Groups Listed by Frequency of Occurrence, page 159). In extreme cases, it may be necessary to attempt Pawley fitting and even structure solution in all possible space groups.
- Appendix D: Frequency of Occurrence of Space Groups (see page 159) lists space groups in decreasing order of their frequency of occurrence in the Cambridge Structural Database. A separate listing is given for chiral space groups, if you know that your structure is enantiomerically pure (see D.2 Chiral (Sohnke) Space Groups Listed by Frequency of Occurrence, page 161).
- Of course, there are space groups that have identical systematic absences. In such cases, the powder diffraction data alone are insufficient to determine the true space group. In principle, the distribution of structure factors can distinguish between centrosymmetric and non-centrosymmetric space groups, but in practice, powder diffraction data are rarely of sufficient quality to permit this distinction.

8 PAWLEY FITTING

8.1 Overview of Pawley Fitting

The aim of Pawley fitting in DASH is to fit the observed powder profile in the absence of a structural model using: (a) a polynomial representing the background, (b) a set of parameters describing peak shape, (c) zero-point and cell-dimension parameters, and (d) estimates of the individual reflection intensities. The overall fit between the resulting calculated profile and the observed profile is displayed graphically and expressed by a number of goodness-of-fit statistics, including c^2. Provided the fit is good enough, the refined reflection intensities can then be used for structure solution.

The final Pawley fit to the data represents the best fit to the data that you can obtain. As such, it serves as a reference value to aim for during the structure solution process. The final Pawley fit chi-squared can be viewed throughout the rest of the structure solution process by selecting **Pawley / SA** from the **View** menu (see Section 2.6.5, page 16).

This section covers how to perform Pawley fitting of your data, including:

- An overview of the usual sequence of steps (see Section 8.2, page 69).
- Truncating the profile (i.e. identifying the 2q value beyond which there is little or no useful Bragg intensity) (see Section 8.4, page 75).
- Selecting and fitting peaks so as to obtain good estimates of peak-shape and cell parameters prior to performing the initial Pawley fit (see Section 8.5, page 79).
- Performing an initial Pawley fit of the background and reflection intensities (see Section 8.6, page 83).
- Improving the fit by refining the cell, zero-point and, possibly, peak-shape parameters (see Section 8.7, page 85).
- Assessing the quality of a Pawley fit (see Section 8.8, page 87).
- Dealing with numerical instabilities (see Section 8.9, page 90).

8.2 Sequence of Operations in Pawley Fitting

The usual sequence of operation in Pawley fitting, as implemented in DASH, is:

- Truncate the data to a suitable range for structure solution (see Section 8.4, page 75).
- Specify a space group and initial values for the cell parameters (see Section 7, page 65). The Pawley fit can be performed in the default space group (i.e. a group with no systematic absences) of the appropriate crystal system, or in the true space group if it is known.
- Select about 8 peaks from across the 2q range, choosing (as far as possible) strong, single reflections (see Section 8.5, page 79). As the peaks are selected, DASH automatically refines the cell dimensions and the peak-shape parameters. Note that the automatic cell refinement does not

commence until sufficient reflections with non-zero values of *h*, *k* and *l* have been sampled.

- Once the program is satisfied with the stability of these parameters, it allows simultaneous refinement of (a) a polynomial representing the background, and (b) the reflection intensities. The cell parameters, zero-point and peak-shape parameters are kept fixed during this stage of the procedure (see Section 8.6, page 83).
- If the fit looks promising, then it is usual to run more cycles of refinement allowing the cell parameters and zero-point to vary (see Section 8.7, page 85).
- Finally, some or all of the peak-shape parameters may be refined, though this is rarely necessary (see Section 8.8, page 87).
- The results of the Pawley refinement (crucially, the reflection intensities and their covariances) can then be saved for use in structure solution (see Section 10, page 101).

8.3 How to Use the Interface for Pawley Fitting

There are two methods for setting up the information needed for the Pawley Refinement:

- The DASH Wizard which will help ensure that items are not forgotten (see Section 2.10, page 21).
- The main Window option, for more experienced DASH users (see Section 8.3.1, page 70).

8.3.1 Using the Main Window to Prepare for Pawley Refinement

- Load an X-ray diffraction powder pattern (see Section 2.2, page 5).
- Subtract the background (see Section 2.4.1, page 9).
- Select **View** from top-level menu.
- Select **Diffraction Setup** from tab bar.
- Input type of data e.g. Synchrotron, Wavelength etc. (see Section 2.6, page 12)
- Click **Apply**.
- Select **Cell Parameters** from tab bar.
- Fill in details of the cell dimensions and space group.
- Click **Apply.**
- Click **OK.**
- Proceed to pick peaks (see Section 5.1, page 47).

8.3.2 Picking of Peaks for Pawley Refinement

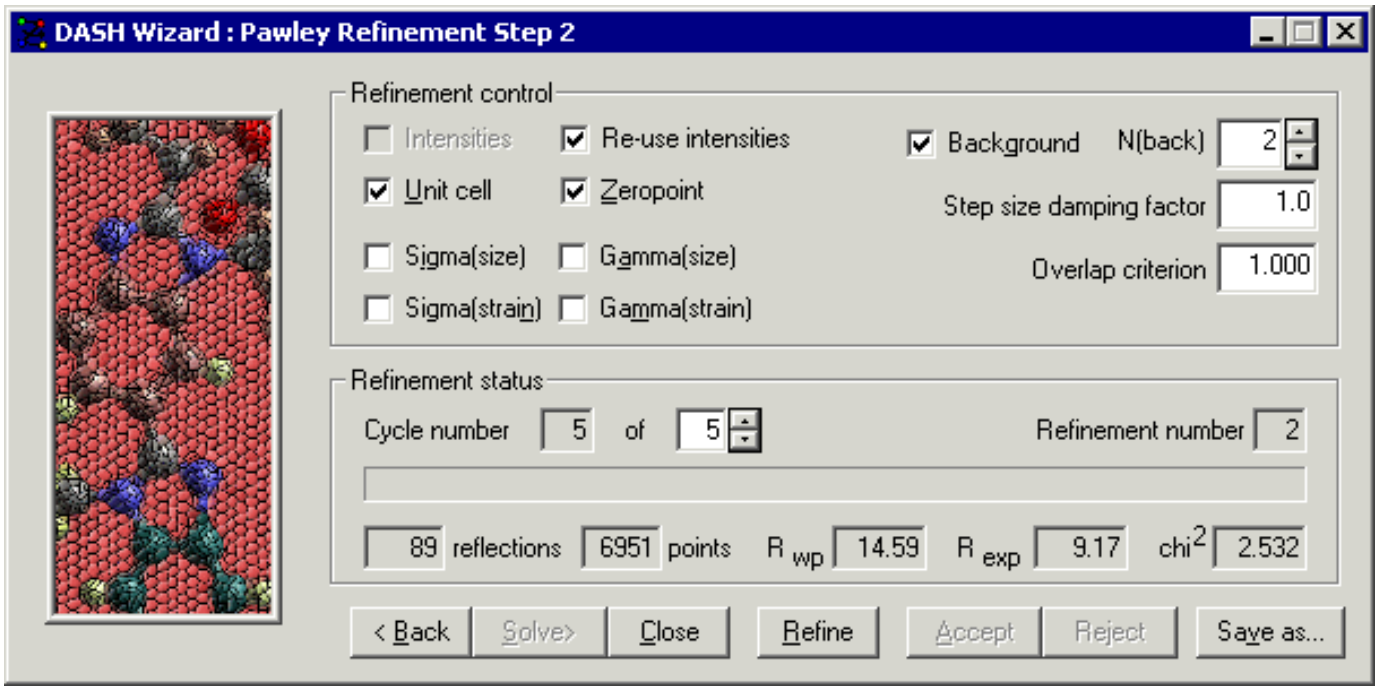
- Zoom in to isolated single peaks, working from low to high 2q (see Section 4.1, page 37).
- Fit the peaks using the right mouse button as described in Section 5.1, page 47.
- Continue picking peaks, remembering that you need to sample a total of about 8 over the whole

2q range.

- When 8 peaks have been fitted a *Pawley Refinement Status* window appears (see Section 8.3.3, page 71).

8.3.3 Pawley Refinement Interface

The *Pawley Refinement Status* window appears by clicking the icon in the main window, or by selecting **Pawley Refinement** from the top-level **Mode** menu.



The options available are:

Refined variables:

- **Intensities**: all Pawley refinements treat the reflection intensities as variables in a least-squares fit.
- **Re-use refined intensities**: DASH utilises the intensities extracted from the previous cycle as a starting point for the next cycle. Deselection of this option causes DASH to ignore the previous values and generate a new set from scratch.
- **Unit Cell:** when selected, the unit cell parameters are refined.
- **Zeropoint**: when selected, the zero-point correction for the diffraction data is refined.
- **Background**: when selected, a polynomial of order shown is fitted to the background (see Section 8.6.1, page 83).
- **N(back)**: the number of terms to be used in the polynomial.
- **Sigma(size)**: when selected, the peak shape parameter sigma-1 is refined (see Section 8.6.2, page 85).

- **Sigma(strain)**: when selected, the peak shape parameter sigma-2 is refined (see Section 8.6.2, page 85).
- **Gamma(size)**: when selected, the peak shape parameter gamma-1 is refined (see Section 8.6.2, page 85).
- **Gamma(strain)**: when selected, the peak shape parameter gamma-2 is refined (see Section 8.6.2, page 85).

Fixed Parameters:
- *Overlap Criterion:* this controls when closely overlapping peaks are treated as a single variable in the Pawley fit, rather than as discrete variables. The default value of 1.0 is sufficient for fitting most data sets.
- *Damping:* Setting this factor to a value of e.g. 0.1 might help stabilise very unstable refinements.

Refinement Status:
The lower section of the window displays the current status of the refinement:

- *Cycle number*: the spinner gives control over the maximum number of cycles of refinement that are performed upon selecting the **Refine** button.
- *Refinement number*: this simply records a sequential number for each refinement that has been run.

The bottom line of boxes reports the results of a refinement run:

- *Reflections*: this is the number of extracted reflection intensities.
- *Points*: this is the number of profile data points used.
- *Rwp*: this is the weighted profile R-factor (see Appendix C: Definitions of DASH Figures of Merit, page 157).
- *R(exp)*: this is the expected profile R-factor (see Appendix C: Definitions of DASH Figures of Merit, page 157).
- *Chi*^{2}: this is the profile c^2 (see Appendix C: Definitions of DASH Figures of Merit, page 157).

Buttons:
- **Refine**: start the Pawley refinement.
- **Close**: close the window.
- **Accept**: accept the results of the refinement that has just completed.
- **Reject**: reject the results of the refinement that has just completed.
- **Save as...** : save the refinement results as a Pawley-Fit file ready for structure solution (see Section 8.3.5, page 74).

- **Solve**: proceed to the Structure Solution stage (see Section 10, page 101).

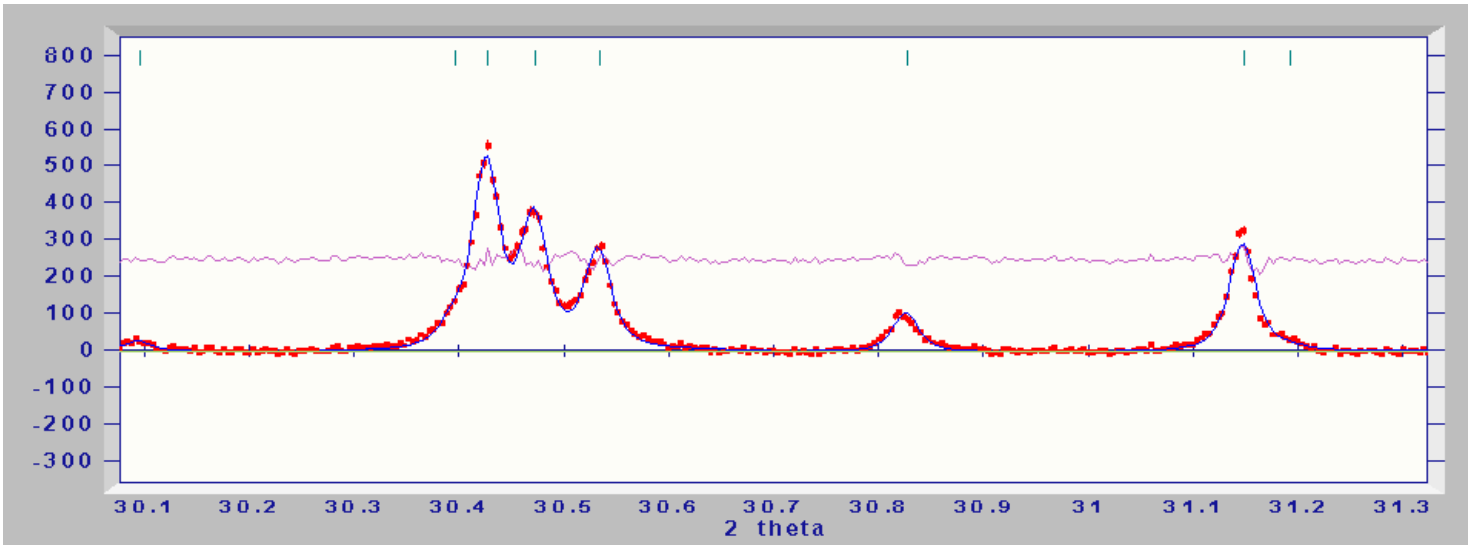
8.3.4 Pawley Refinement: an Example

Using the *Example.xye* file, the unit cell parameters and space group information, and having selected 8 peaks (see Section 8.3.2, page 70) you will arrive at a window as shown in Section 8.3.3, page 71.

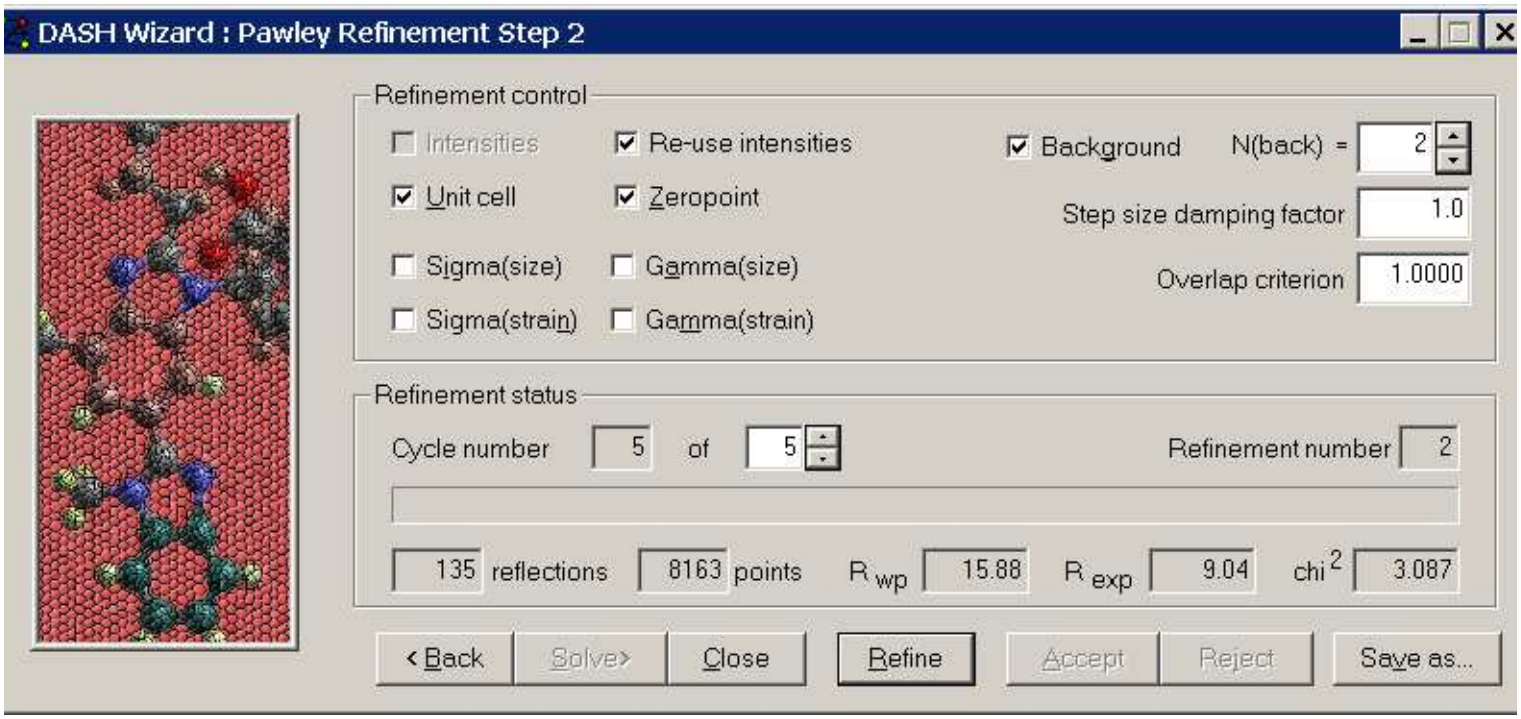
- In this example, we have assumed that the background has been fitted by the Monte-Carlo background subtraction routine. If the background had not already been subtracted, the only difference would be that **N(back)** would be automatically set to 10.
- The data has been truncated to a resolution of 2.0 Å.
- In the initial Pawley refinement, only the terms describing the background and the terms corresponding to individual reflection intensities are refined, using the previously refined unit cell and zero-point.
- When you select **Refine** 3 cycles of least squares are performed.
- This should return figures similar to the ones given below:

  89 reflections 6950 points R_{wp}= 33.52 R_{exp} = 9.22 c^2 = 13.210

- Select **Accept** to accept the results of this refinement, the fit is displayed.
- Now click in the main window and select **Home** to see how well the data are fitted. The (observed minus calculated) plot is shown in pink and emphasises any misfit in the data. If you look closely at the data, you are likely to see something like this:



The next stage is to refine **Background**, **Intensities, Unit Cell** and **Zeropoint**. DASH assumes that after the initial background and intensities fit, you will automatically want to refine the unit cell and zero-point. Accordingly, tick marks are automatically set in the menu boxes. Normally 5 cycles of refinement are sufficient. Click the **Refine** button and then the **Accept** button to store the results of refinement. The *Pawley Refinement Status* window now looks like this:

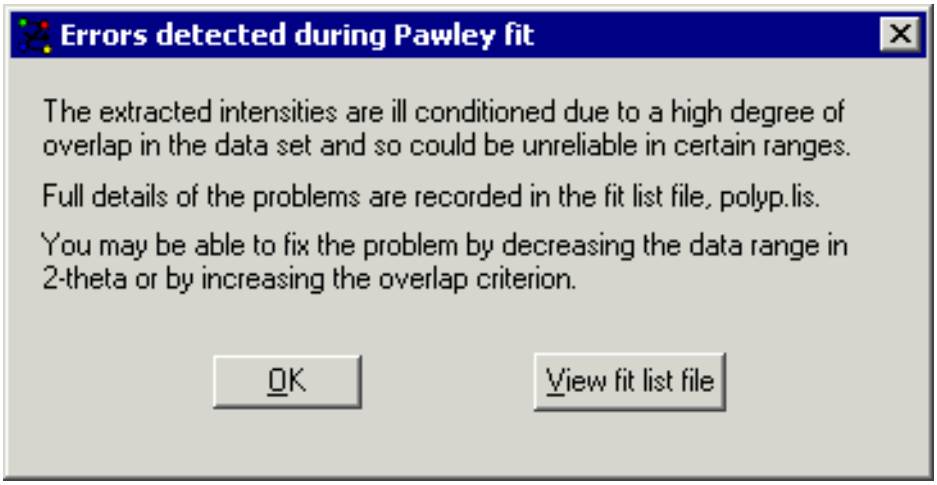


8.3.5 Saving the Results of Pawley Refinement (Pawley-Fit files)

- In the example shown in Section 8.3.4, page 73 the c^2 of 3.087 is very good, so you would then save the results of this refinement as the basis for structure solution.
- Select **Accept** and then **Save as...**; the *Save Diffraction Information for structure solution* window appears into which you can enter a file name, e.g. *Example.sdi*; click **Save**.
- You can save the results of several independent Pawley refinements, each in its own Pawley-Fit file, with extension `.sdi`.

8.3.6 Mathematical Problems with Pawley Refinement

For poor quality data sets there can be problems in fitting the background with the polynomial mathematical procedure. When this happens an *Errors detected during Pawley fit* window will appear:



View fit list file shows the output of the fitting program. If you look at the last few lines of this file there are messages as to why the procedure failed. Do not accept the results of this refinement. Check the following:

- 2q range: consider if there is really any useful data above a certain 2q, and truncate(see Section 8.4, page 75).
- *Overlap Criterion*: it is always worthwhile re-running the refinement with a larger value of the Overlap Criterion (see Section 8.3.3, page 71). For heavily overlapped, weak data, a value of 2.0 may suffice to stabilise the refinement.

8.4 Truncating the Data

- DASH limits the number of reflections that can be refined in a Pawley refinement to around 350. This is all that you will need to solve the majority of organic structures. Accordingly, it may be necessary to truncate the data, i.e. throw away counts above a certain 2q value.
- The actual truncation of the data must be done before the Pawley refinement stage, either by manually editing the input data file or by using the Wizard.
- You must judge the point in the profile at which the significant information ends.
- As a general rule, if data can be used up to 1.5 Å, there will generally be enough information to solve most organic structures; i.e. for CuK_{a1} radiation (wavelength l = 1.54056 Å):

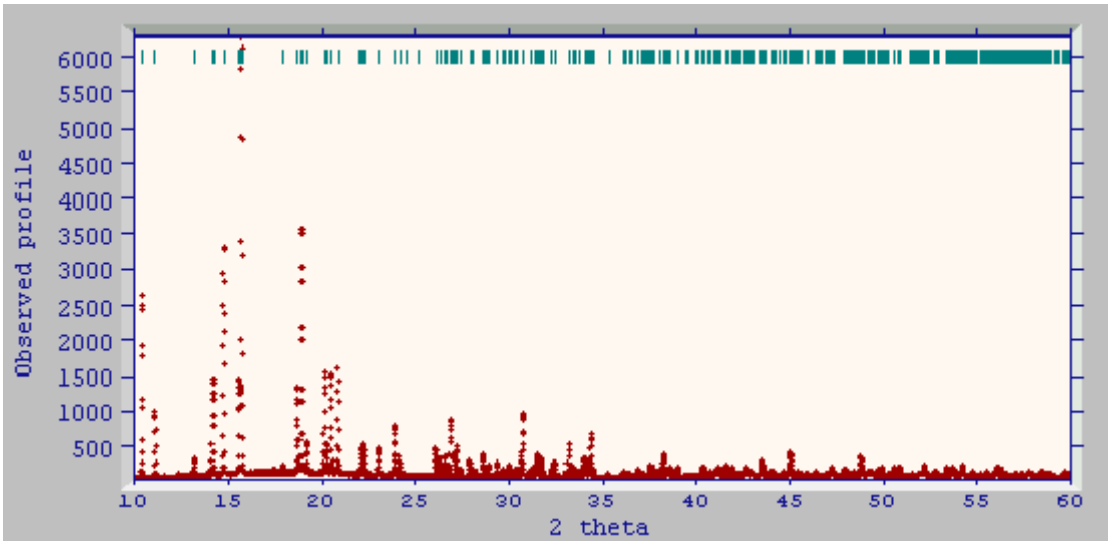
  q = sin-1(l/2d)
  Therefore:
  q = sin-1(1.54056/ (2 * 1.5)) @30^o
  Therefore:
  2q @60^o

Sometimes, the powder pattern will not contain useful data to this high angle. In such cases, it is better to cut the data down to lower resolution e.g. 2.0 Å or even 2.5 Å.

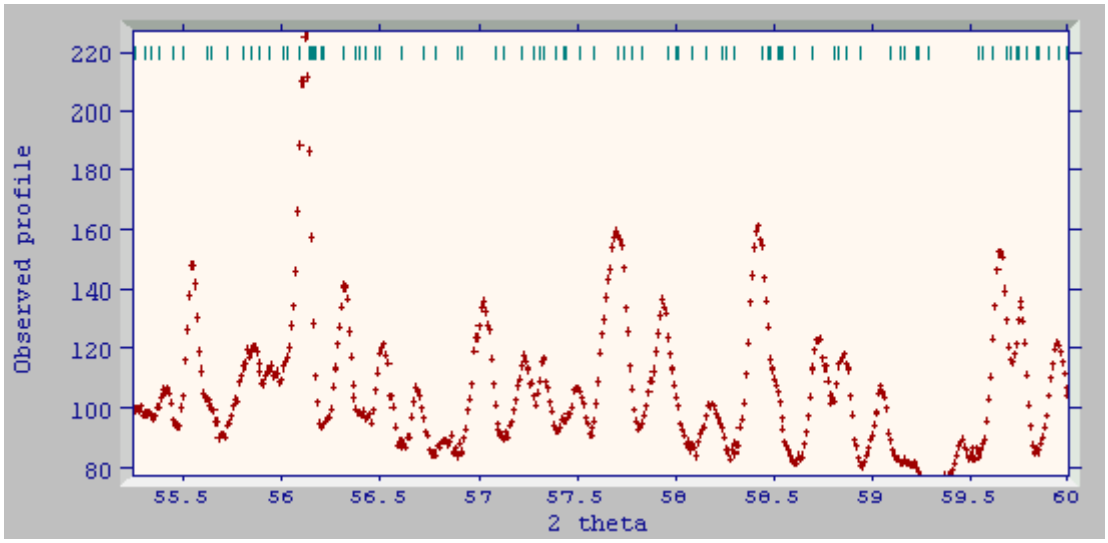
- Examples are provided illustrating some suitable cut-off points for:
  - A high resolution profile (see Section 8.4.1, page 76).
  - A medium resolution profile (see Section 8.4.2, page 77).
  - A low resolution profile (see Section 8.4.3, page 78).

8.4.1 Truncating High Resolution Data: an Example

- Synchrotron dataset, with an incident wavelength of 1.1 Å; thus data were collected to maximum $2\theta = 60^{o}$, equating to 1.1 Å spatial resolution:



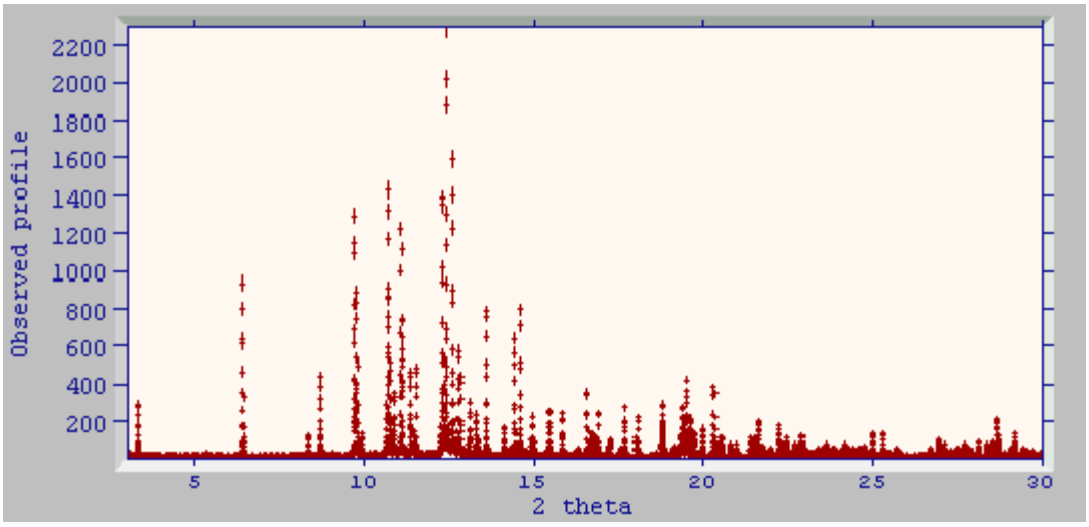
- There are 418 reflections in this data range, which is slightly more than DASH will handle by default. However, a significant proportion (nearly 25%) of these reflections occur in the last 5% of data:



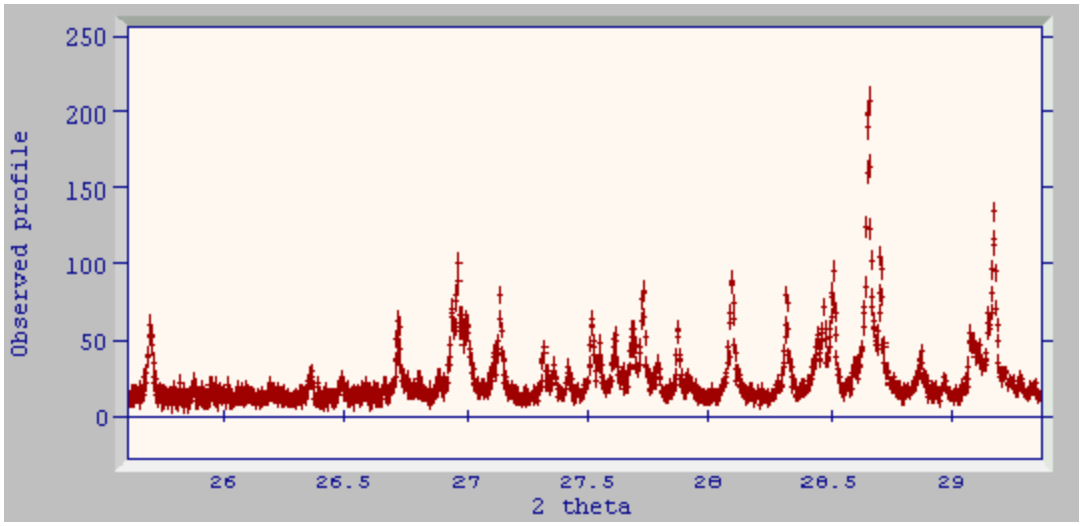
- Although the crystal is still diffracting quite strongly at this point (sufficiently well for the information content to be useful in structure refinement) it is clear that the extent of reflection overlap is high. If we cut the data limit back 5° to 55°, we simplify the problem by reducing the number of reflections to be refined to only 323, at the cost of only 0.1 Å loss in spatial resolution. In fact, this structure can easily be solved from data extending to 1.0 Å resolution (43° 2q) and the total number of reflections in this range is then only 163.

8.4.2 Truncating Medium Resolution Data: an Example

- Synchrotron dataset with an incident wavelength of 0.85 Å, so 1.5 Å resolution equates to around 2q = 33°



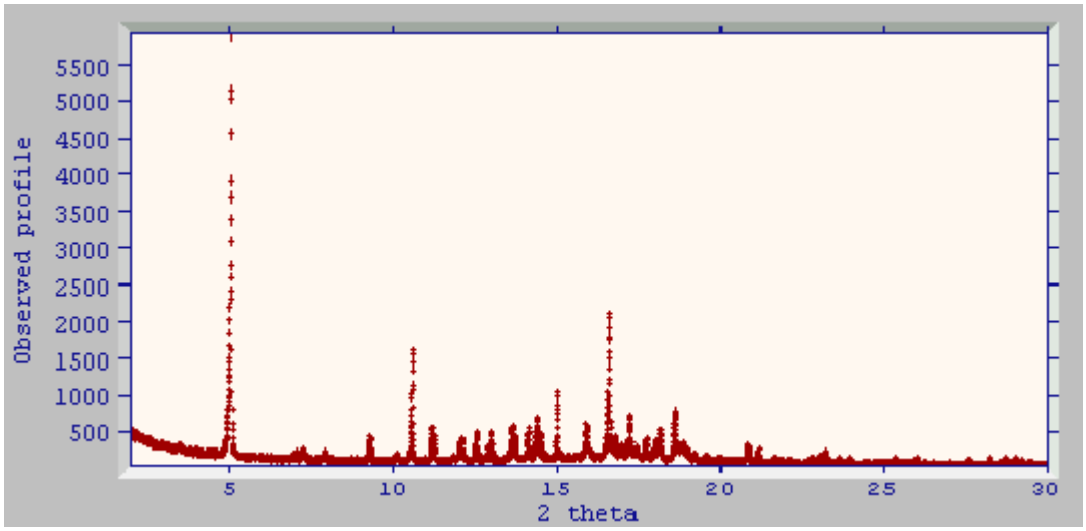
- It is clear that diffraction is still strong at the high-angle end:



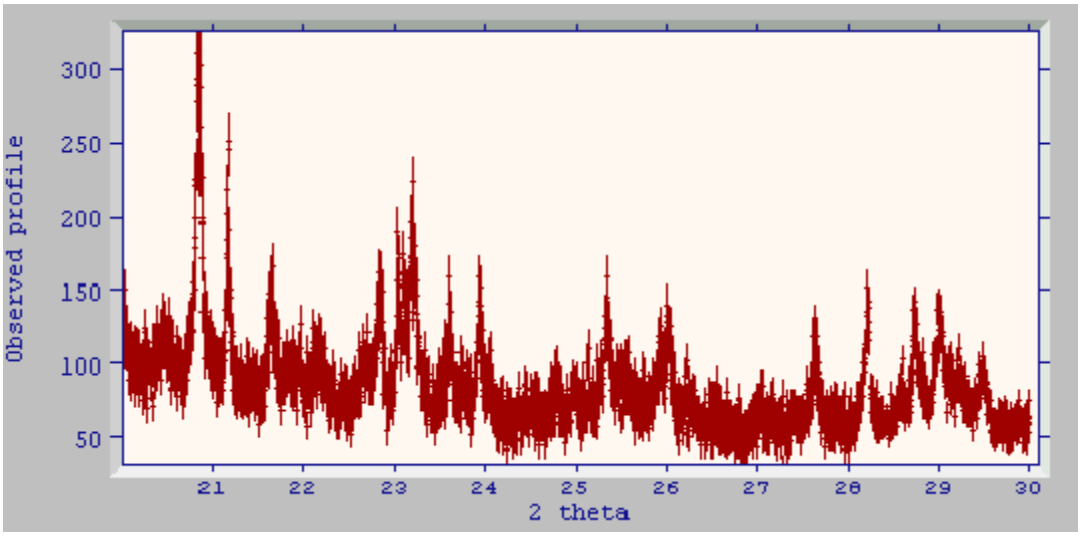
- There are around 350 reflections in the full data range and this can easily be fitted, so truncation is not necessary.

8.4.3 Truncating Low Resolution Data: an Example

- Synchrotron dataset with an incident wavelength of 1.15 Å; thus, 1.5 Å spatial resolution equates to a 2q value of 45°. However, it is clear that the diffraction data is fading long before this point:



- Given the poor signal to noise ratio, there is little point in fitting the data beyond about 30°, which equates to about 2.2 Å resolution (this is sufficient to solve the structure):



8.5 Choosing Peaks Prior to Initial Pawley Fitting

The first step in Pawley fitting is to select some peaks for refining the peak-shape parameters and the unit cell dimensions. Once enough peaks have been selected (usually 8 to 10), and stable estimates of these parameters have been obtained, an initial Pawley refinement of the background and reflection intensities can be performed. Guidelines for peak selection are:

- If possible, choose strong, well-defined reflections that, collectively, span a broad 2q range.
- They should include at least one or two peaks at low 2q, so that any low-angle peak asymmetry is well described in the refinement of the peak-shape parameters.
- If possible, isolated reflections should be chosen. These can be identified by looking at the tick marks at the top of the display, which show the reflection positions calculated from the cell and space group you have specified. However, it is valid to fit multiple peaks if necessary.
- As selection of peaks proceeds, DASH will update peak shape parameters, and then cell parameters, and then indicate that it is ready to perform the initial Pawley refinement.

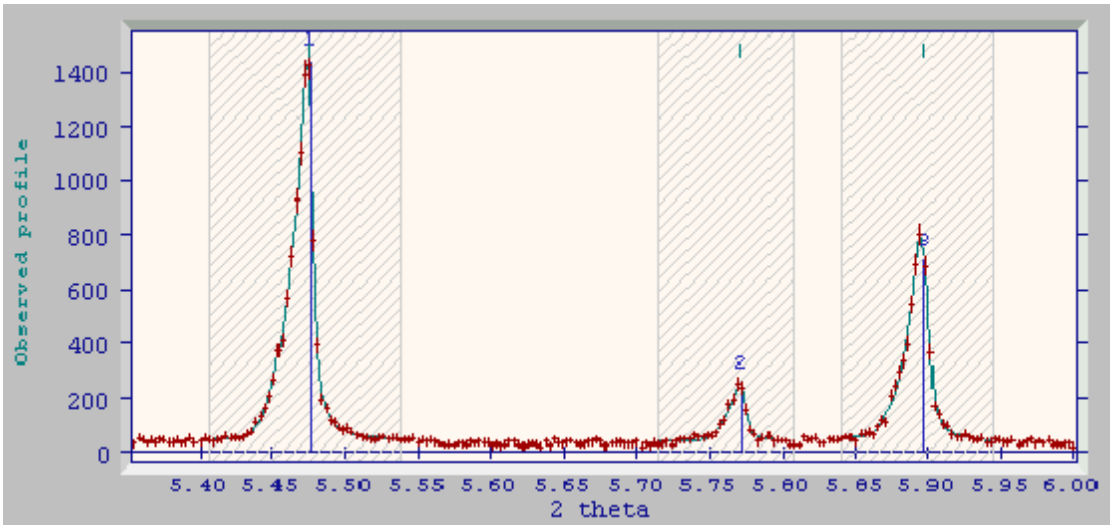
8.5.1 Choosing Peaks Prior to Initial Pawley Fitting: an Example (see page 79)

8.5.1 Choosing Peaks Prior to Initial Pawley Fitting: an Example

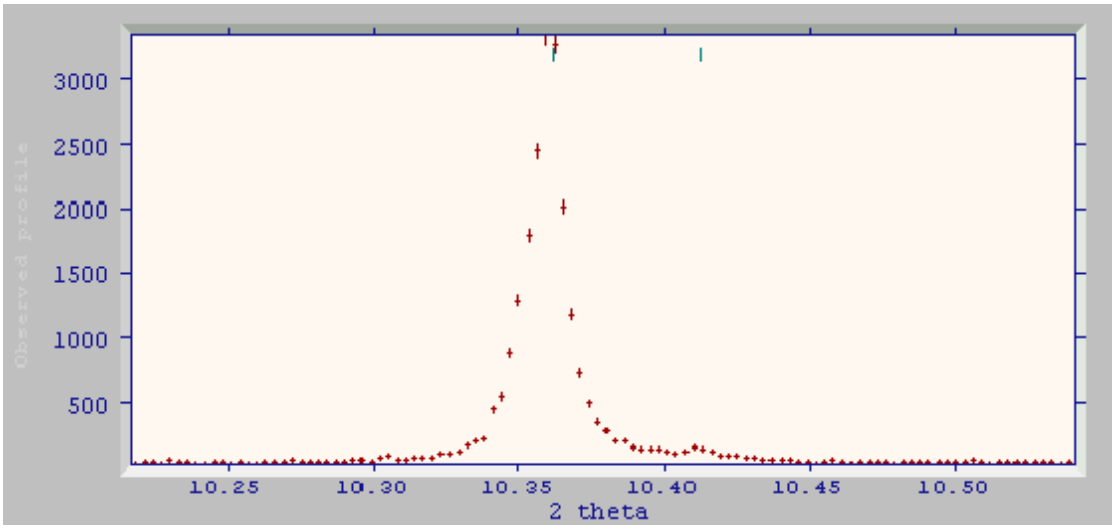
The following example of peak selection for Pawley refinement illustrates some of the situations you will encounter:

- Fitting the first three peaks in the pattern. These peaks are all fairly strong and well separated and can be fitted easily. Inclusion of these peaks helps subsequent cell refinement (as they correspond to low order reflections) and gives a good parameterisation of any asymmetry

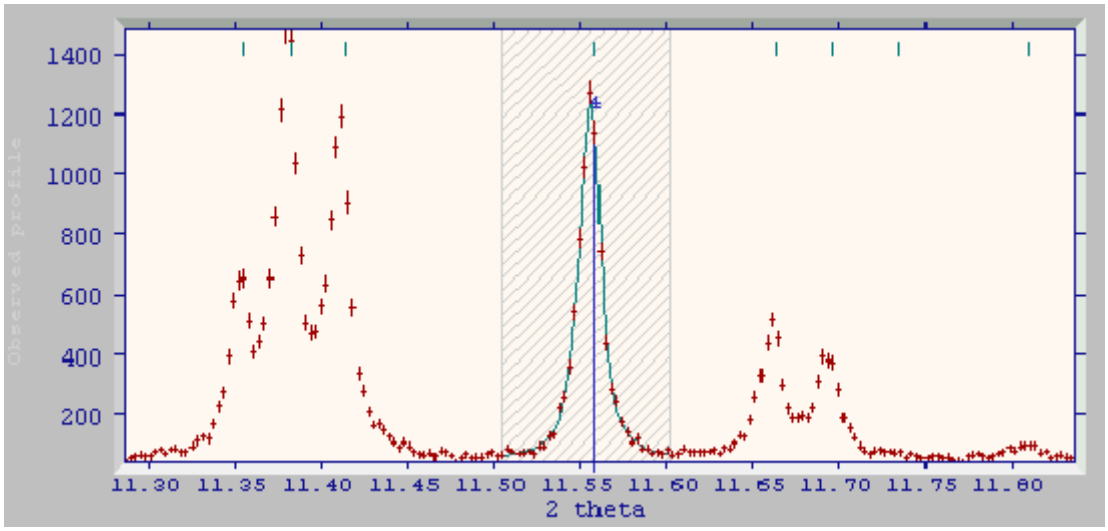
present:



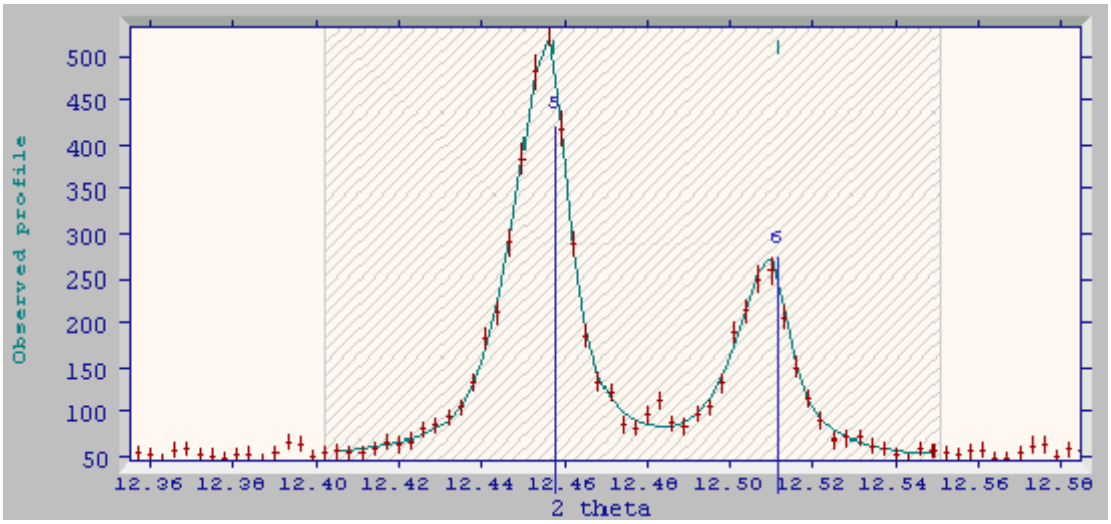
- Looking further up the pattern, there is a strong peak that could be selected, but it has a weak satellite peak to the right, which would need to be fitted simultaneously. The weak peak is not strong enough to provide useful peak parameterisation information in its own right, but it is strong enough to affect parameterisation of the strong peak, so would have to be included in the fit. There are almost certainly better choices at other points in the pattern, so do not use these peaks:



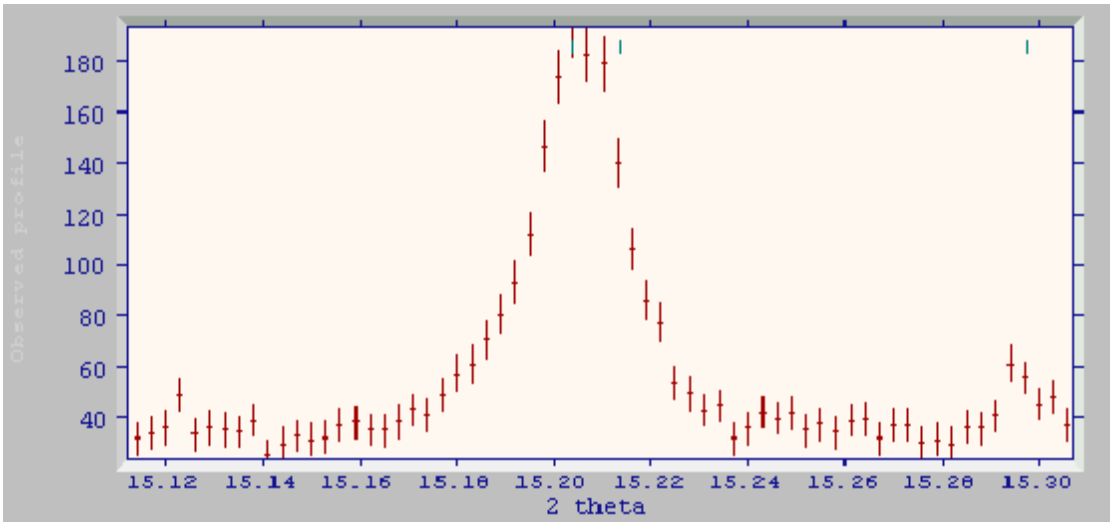
- Still further up the pattern, there is a strong isolated peak that can easily be used. The triplet and the doublet on either side of it could be selected too, but we really want to sample peaks throughout the 2q range in order to parameterise the peak shape right across the pattern. By fitting the triplet, we simply get three peaks telling us about the local peak shape around 2q = 11.37^{o}. Fitting the single peak at ~11.56^{o} gives us exactly the same information:



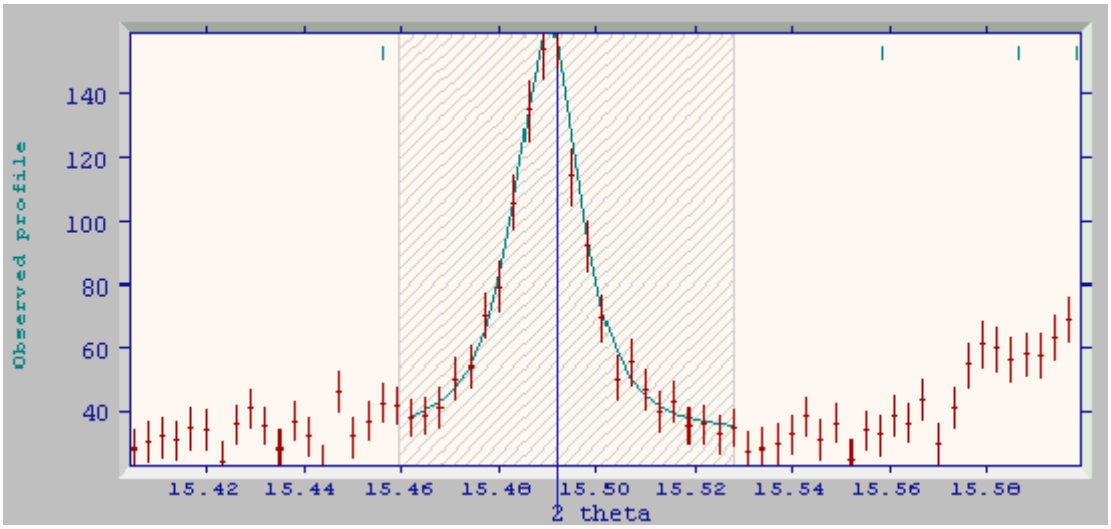
- Even further up the pattern, there is a nicely isolated pair of fairly strong peaks. We could fit these reflections individually, but as they overlap a little, it is better to fit them together as a doublet:



- Note that these two peaks contribute two entries in the list of peak positions (select **Peak Positions** from the **View** menu) yet only a single entry in the lists of peak shape parameters (select **Peak Widths** from the **View** menu) as both peaks have been fitted with the same values for sigma and gamma.
- Moving further up again, there is a peak that has contributions from two Bragg reflections (i.e. there are two tick-marks close together above the peak), but there are no clues as to their exact relative positions. Peaks such as this are best avoided because, each time a peak is fitted, DASH attempts to refine the input unit cell (the one you obtained by indexing, and entered at the start of the Pawley fitting process). In this situation, the peak positions are not well defined and there is a risk that the cell could be refined away from the correct values. Thus, avoid:



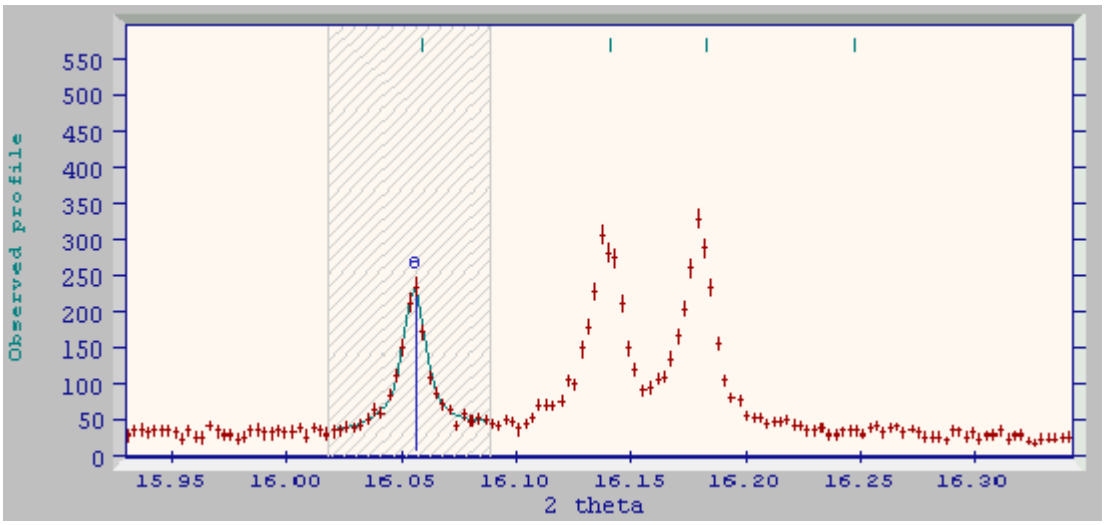
- Next, we find a moderately strong reflection which is sufficiently isolated to allow fitting, even with a narrow selection range. The very weak peak on the left is not strong enough to interfere:



- This is the 7th peak selected; thus we have accurate peak shape descriptors and peak positions for 7 peaks throughout the pattern. This is sufficient information for a least-squares cell refinement to be performed on this monoclinic cell, and DASH will do this automatically. A sign that lattice parameter refinement has commenced is that the tick mark above the peak *jumps* closer to the peak after fitting; this indicates that the cell has been updated to incorporate the latest peak position.

- DASH has automatic settings that determine the best point at which to attempt a full Pawley refinement. At this time, it automatically displays the *Pawley Refinement Status* window. In this example, the window is not yet displayed, so we continue picking peaks.

- Further up the pattern, there is another isolated peak which is easily fitted. Now, with a total of 8 peaks fitted, the *Pawley Refinement Status* window is displayed automatically.



8.6 Initial Pawley Fitting of Intensities and Background

- Once enough peaks have been selected and the program has stable estimates of the cell dimensions and peak-shape parameters, you can perform an initial Pawley fit of the reflection intensities and the background. This will be indicated by the appearance of a pop-up window.
- Usually, you should keep the cell dimensions and peak-shape parameters fixed in this initial refinement. Allowing them to vary is likely to destabilise the refinement.
- The following subsections cover:

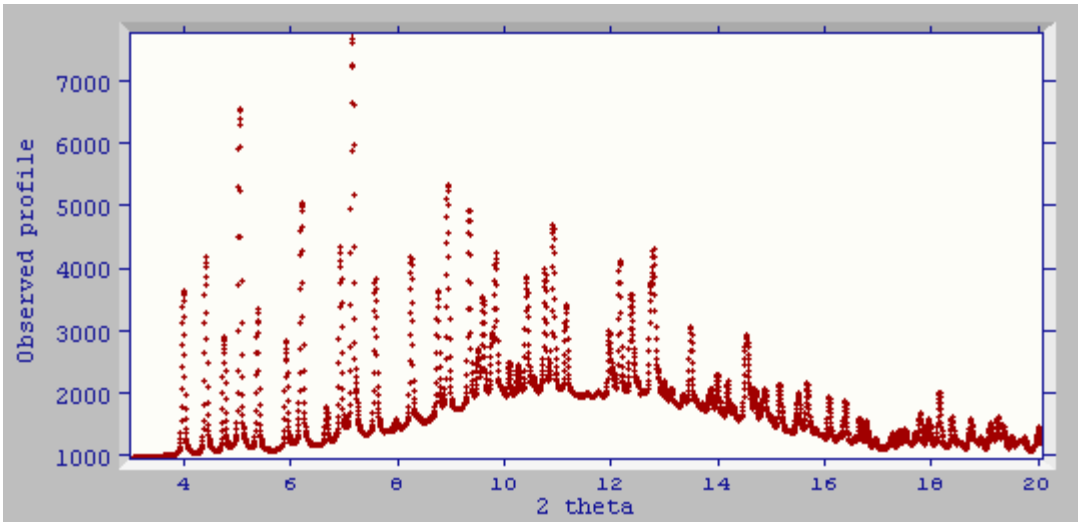
  - Background fitting (see Section 8.6.1, page 83).
  - Reflection-intensity fitting (see Section 8.6.2, page 85).

8.6.1 Background Fitting of Raw Data in the Pawley Refinement

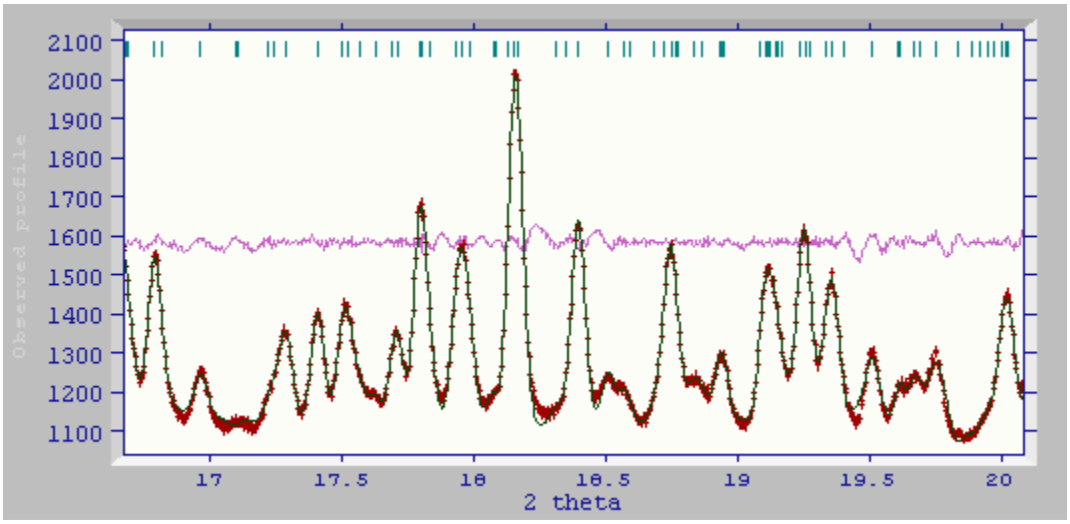
- Note that in this example, we have assumed that the background has not been fitted by the Monte-Carlo background subtraction routine. This is by way of illustrating use of the Pawley refinement for a raw data set.
- Background intensity is primarily due to amorphous content in the sample and scattering from experimental components such as a glass capillary or cryostat chamber.
- The background is fitted to a polynomial which, by default, has 10 terms.
- It is undesirable to use more background terms than necessary to describe the data, since high order polynomials can begin modelling genuine peak intensity. This leads to high correlations between the background and peak intensities, especially at high angles.
- A polynomial with as few as 5 terms might be enough for a flat or gently curving background; at

the other extreme, you should never have to use more than about 15. Normally, the simplest policy is to try the default of 10 and accept it if the fit looks satisfactory.

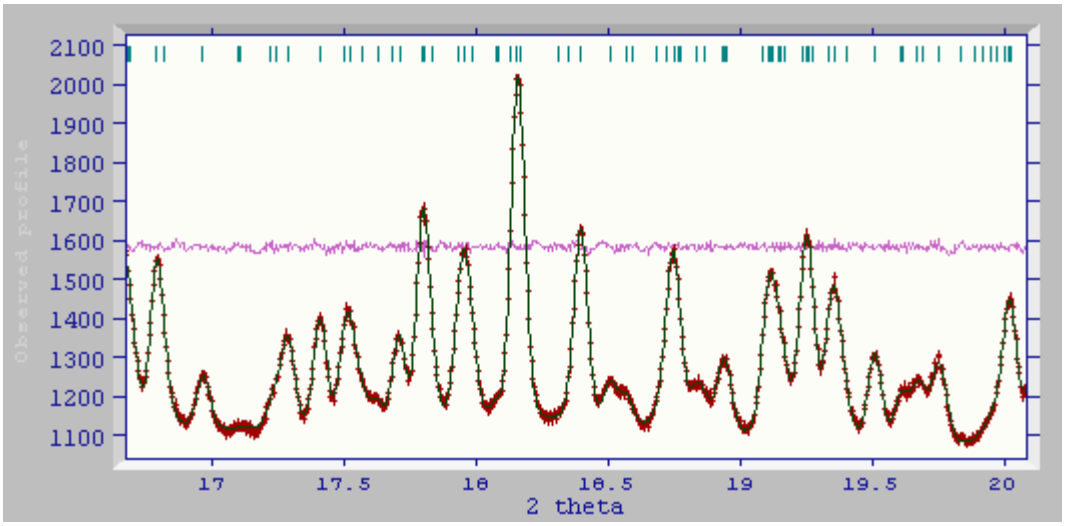
- The following example profile has a very non-uniform background:



- In this case, a total of 10 background terms were used and the Pawley fit returned R_{wp}=5.9, R_{exp}=2.5 and c^2 = 5.6. Some misfit (though not much) is evident between the peaks at high angles:



- Increasing the number of background terms to 15 brings about a significant improvement, with R_{wp}=5.65, R_{exp}=2.5 and c^2 = 5.15:



- A further increase to 20 background terms improves the fit slightly, but the high angle plot is virtually indistinguishable from that with 15 parameters, so the gains in going to 20th order are not worth it (In fact, the fit with a 10th order polynomial is sufficient to solve the structure).

8.6.2 Reflection-Intensity Fitting

- In fitting the individual reflection intensities, a peak shape description function is centred at the calculated 2q of each reflection. The parameters used in the function (s_1, s_2, g_1, g_2) have already been determined from the initial set of peaks you selected and should not be varied at this stage. They can be refined later, once the unit cell and zero-point have been fully optimised.
- The fitting procedure not only estimates the individual reflection intensities but also their covariances.
- If a group of reflections are too close together, then the observed intensity for that clump of reflections is treated as a single variable, with the resultant intensity partitioned equally between the component reflections.
- Look at the output file *polyp.lis* to get information about which reflections have been merged together in this way. The file also lists the total number of reflections and other diagnostic information.

8.7 Hints for Improving the Pawley Fit

Once a reasonable initial Pawley fit has been obtained, it can usually be improved by:

- Adding into the refinement the cell dimensions and zero-point (see Section 8.7.1, page 86).
- Refining some or all of the peak-shape parameters (see Section 8.7.2, page 86).

8.7.1 Cell Dimension and Zero-Point Refinement

An initial Pawley fit can almost always be improved by adding into the refinement the cell dimensions and zero-point.

- The cell dimensions will now be refined against the whole of the profile rather than the small number of peaks you chose initially.
- The zero-point (origin of 2θ axis) may have been measured experimentally for the instrument on which data were collected, but it is always desirable to refine it anyway. A refined absolute value of 0.01^{o} would not be atypical.
- An error in the zero-point may manifest itself on the profile difference plot by a systematic shift in peak positions.

8.7.2 Peak Shape Refinement

- Unless and until the peak-shape parameters are explicitly included in the Pawley refinement, their values will be based only on the few peaks you chose when setting up the initial refinement.
- Normally, you should not need to refine the peak shapes further. However, if you notice that certain peaks are not well fitted, it may be worth including them directly in the peak shape estimation process. Sweep out an area to select the peak and fit as before. The overall peak shape description will be updated and you can re-run the Pawley fit with the updated parameters.
- The peak shape used within DASH is a full Voigt function (a convolution of a Lorentzian and a Gaussian function) which uses 2 parameters (s_1 and s_2) to describe the angle-dependent Gaussian component:

  $$s^2 = s_1^2 \sec^2\theta + s_2^2 \tan^2\theta$$

  and 2 parameters (g_1 and g_2) to describe the angle-dependent Lorentzian component:

  $$g = g_1 \sec\theta + g_2 \tan\theta$$

  There are also two asymmetry parameters, HPSL and HMSL, but these are fully defined by the peak fitting procedure and cannot be refined during the Pawley fitting.
- The refined values of the peak-shape parameters may be seen by selecting **Peak Widths** from the **View** menu. The values should all be positive, though small negative values are occasionally obtained.
- There may be some cases (usually those that involve some anisotropic line broadening) in which it is useful to refine the peak parameters within the least squares of the Pawley fit. In general, only peak shape parameters of sizeable magnitude should be refined; there is little to be gained by refining a single parameter that is very close to zero, as its contribution to the overall magnitude of the composite width will be negligible. Refinement of small peak shape

parameters may lead to numerical instabilities.

8.8 Assessing the Quality of the Pawley Fit

The quality of a Pawley fit must be judged in two different ways, both of which are important:

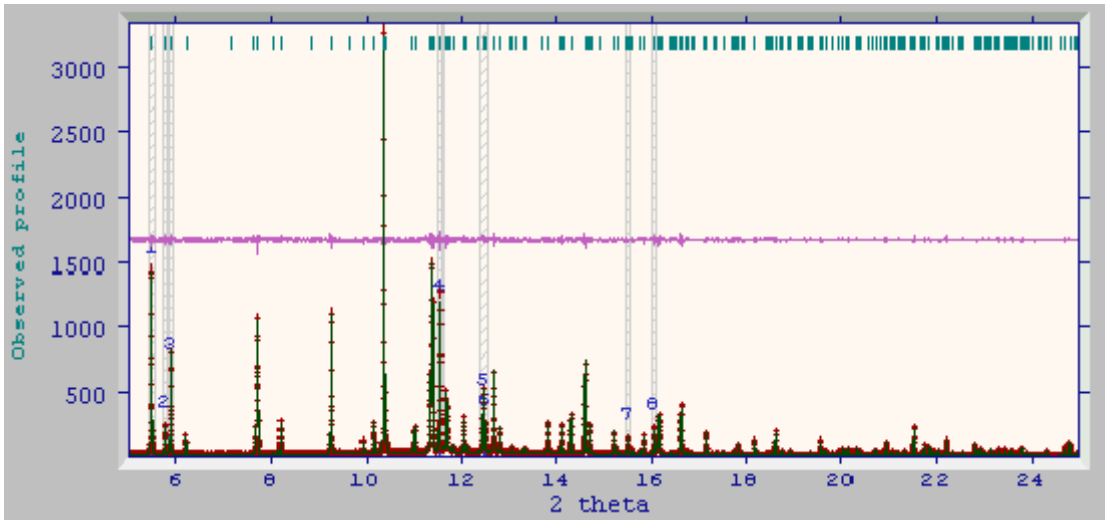
- How good are the goodness-of-fit parameters ? (see Section 8.8.1, page 87)
- How do the observed and fitted profiles compare visually ? (see Section 8.8.2, page 87)

8.8.1 Interpreting Pawley Fit Parameters

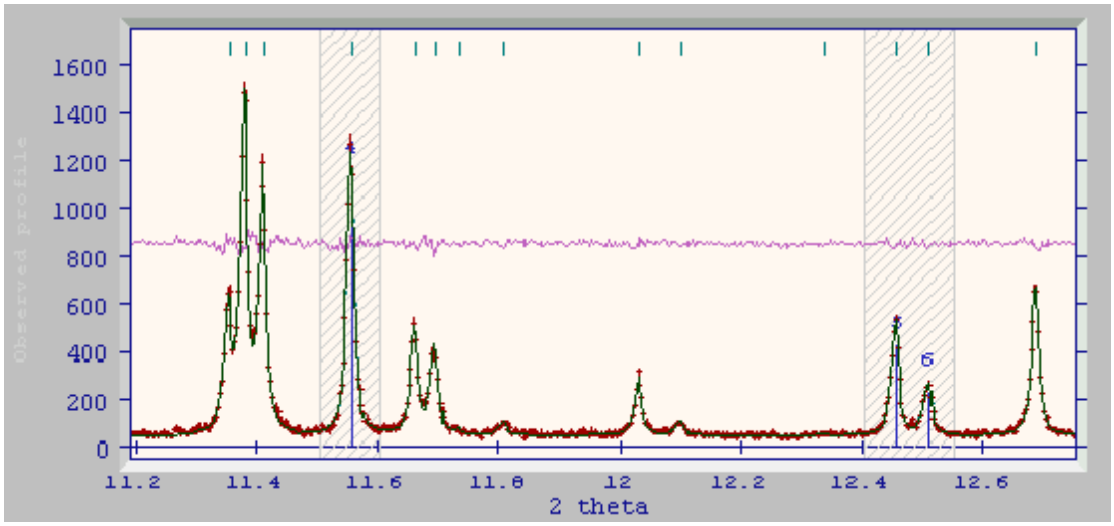
- The parameters R_{wp}, R_{exp} and c^2 are a guide to the quality of a Pawley fit (see Appendix C: Definitions of DASH Figures of Merit, page 157).
- Ideally, R_{wp} should be close to R_{exp} and c^2 should be close to 1.0.
- However, this ideal is often not met in practice, particularly before the cell and zero-point have been refined, and particularly with laboratory data. For example, four recent Pawley fits in a laboratory gave c^2 values of about 1, 5, 15 and 30. These were all synchrotron data sets; generally you should expect higher c^2 values with laboratory data.
- A $c^2 < 1.0$ indicates that the esds on the data set are not quite correct (specifically, they have been overestimated).
- You should not rely solely on the fit parameters; visual inspection of the fit is essential.

8.8.2 Visual Assessment of Pawley Fit

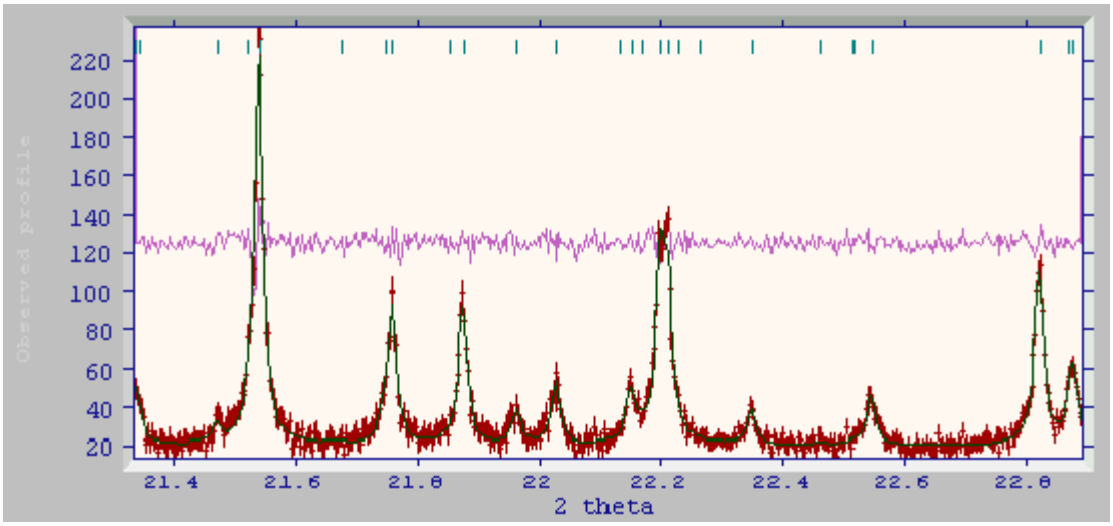
- There is no substitute for examining by eye the fit of observed and calculated profiles; figures of merit which pertain to the entire pattern do not highlight local problems, such as peaks that might not be fitted at all (e.g. impurity peaks).
- The following example shows a good fit. The pink *difference* plot shows no marked areas of misfit:



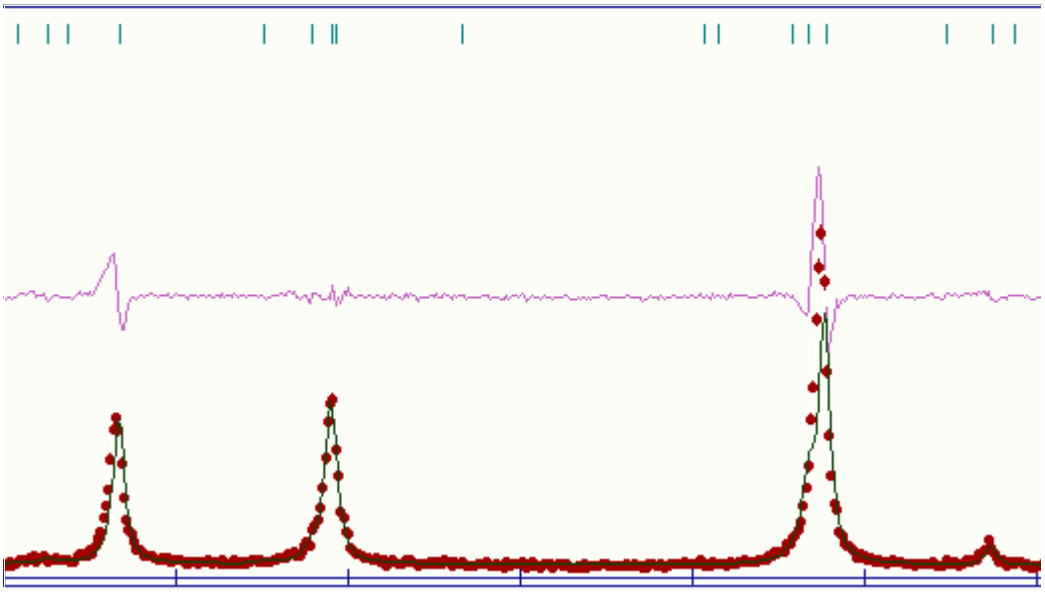
- Zooming in on the fit to the data in the mid range of the pattern shows an excellent fit to the data:



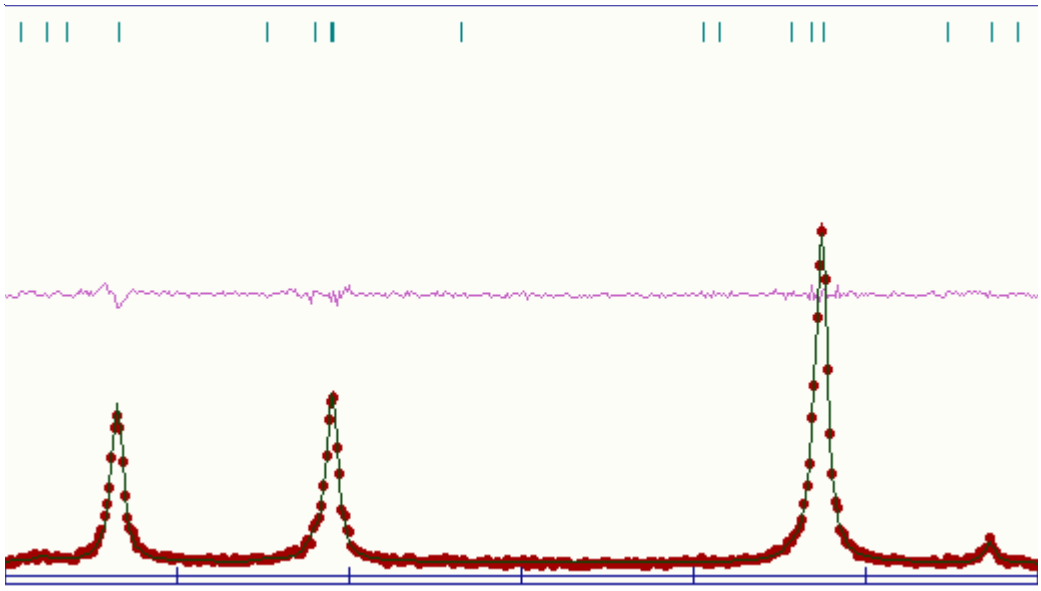
- The same is true at high angle:



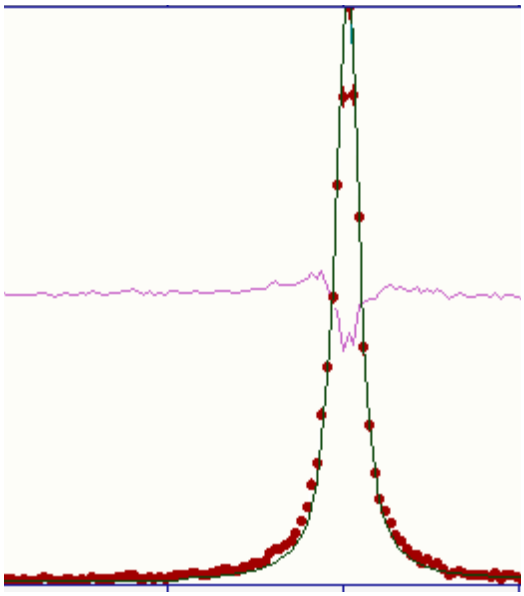
- If the positions of calculated and observed peaks do not match well (e.g. because the zero-point and unit cell are not properly refined), there is a characteristic sinusoidal difference plot:



- In this example, the problem is solved by refining the cell and zero-point:



- Slightly too narrow peak widths result in a *rise; dip; rise* difference plot. This is often seen as a weak trace around very strong peaks:



- A small misfit in tails is not serious: you should start to worry if the misfit is somewhere approaches one-third of the peak height.
- If a *dip*; *rise*; *dip* difference signature is seen, the calculated peak is too wide.

8.9 Numerical Instability in Pawley Refinement

Sometimes, a Pawley refinement will diverge and DASH will display an error message. Possible ways of removing the numerical instability include:

- Truncating the data a bit more (see Section 8.4, page 75).
- Reducing the number of background parameters.
- Taking advantage of the automatic background subtraction that DASH offers (see Section 2.4.1, page 9).

9 BUILDING AND CONSTRAINING MOLECULES

9.1 Overview of Building and Constraining Molecules

DASH solves structures by taking a model of the molecule (or molecules) in the asymmetric unit and moving it (them) around, subject to the constraints of space-group symmetry, until it finds a good match between calculated intensities and those derived from the Pawley fitting. If necessary, rotatable torsion angles in the molecule(s) are allowed to vary, either through a complete 360^{o} range or through a smaller range defined by the user. If the asymmetric unit contains more than one molecule, they are moved independently of one another.

A requirement for solving structures, therefore, is to input appropriate 3D models of the molecule(s). Important things to consider are:

- Building molecules in third-party programs (see Section 9.2, page 93).
- Converting molecules to Z-matrices (see Section 9.4, page 95).
- Reading molecules into DASH and defining the ranges through which bonds can be rotated (see Section 9.5, page 96).
- Treatment of rings (see Section 9.6, page 96).
- Treatment of stereoisomers (see Section 9.7, page 97).
- Molecules on special positions (see Section 9.8, page 98).
- Structures with more than one molecule in the asymmetric unit (see Section 9.9, page 98).

9.2 Building Molecules in Third-Party Programs

There are several programs available for building 3D models of molecules, so DASH does not provide this capability (see Appendix B: Programs for Building 3D Molecules, page 157). Points to remember when building molecules are:

- Ensure that the bond lengths, bond angles and ring conformations have reasonable values, since they will not normally be allowed to vary during simulated annealing. This is most easily achieved by using a fast force-field type minimisation in a modelling package.
- Tables of standard bond lengths may be found in Volume C of *International Tables for X-Ray Crystallography.*
- Torsion angles around single, acyclic bonds can usually be set to any value, since they will be varied during the simulated annealing process.
- Stereochemistries will not be altered during simulated annealing, so if a molecule has more than one chiral centre, it is important that the relative stereochemistries are correct. (If you do not know what is correct, there may be no choice other than to try simulated annealing with each possibility in turn). Absolute stereochemistry does not matter.
- The positions of hydrogen atoms will make little difference to fitting X-ray powder patterns, so

protonation states and torsion angles involving H atoms are not critical. (In practice you may often omit H-atoms entirely.)

- Write molecules out as mol or mol2 files. These can then be read into the DASH program for conversion into the Z-matrix format that DASH requires (see Section 9.4.1, page 95).
- Check the geometry of similar molecules in the CSD (see Section 9.3, page 94).

9.3 Using the Cambridge Structural Database (CSD) to Check Models

- Most molecular model building programs start from a user-created 2D diagram with bond types, from which to construct an approximate 3D model. This is then minimised from this starting point using various force-fields at whatever level of sophistication is available in the program.
- For many molecules there will be no ambiguity as to the final 3D-model as regards the rigid portions, and the settings of any flexible torsion angles will not matter as DASH will recognise these and automatically set these as variable parameters in the structure solution search.
- However, when ring systems are involved, or unusual combinations of elements in functional groups, the user is strongly advised to check for similar molecules in the Cambridge Structural Database (CSD), using the *ConQuest* search program. For example, this may reveal that a particular ring conformation is favoured in the experimental structures, and so one can adjust the 3D-molecular model accordingly.
- It is worthwhile checking the bond lengths and bond angles for any unusual groups for significant deviation from the CSD average. It is probably wiser in such cases to construct the first trial model by taking accurate CSD values than to trust results from force-field energy minimisation. Indeed, for metal complexes the CSD examples are almost essential for good model building.
- Torsion angle distributions may be easily obtained from the CSD using the ConQuest program, and searching on the appropriate fragments, or by using the direct link to *Mogul*, a molecular geometry database, from DASH (Mogul forms part of the *CSD System* which is available from the *CCDC*). The user may decide to reduce the flexibility of the model in DASH during the solution search by placing limits in the torsion angle ranges, or even fixing at certain values as in cases of intramolecular H-bonding.
- In cases of ions such as chloride, there is much information in the CSD knowledge base, *IsoStar*, of intermolecular group…group interactions. This can be used in certain cases to predict the likely distance of an ion from a group in the main molecule, and can greatly improve the chances of solution.
- H-bonding motifs may be important in certain structures with more than one molecule per asymmetric unit. It may be possible to find examples in CSD which would allow one with confidence to fix the relation ship of the second molecule to the first by H-bonding, e.g. carboxylic acid centrosymmetric dimers, or chains with expected geometry.

In summary, use the CSD to check:

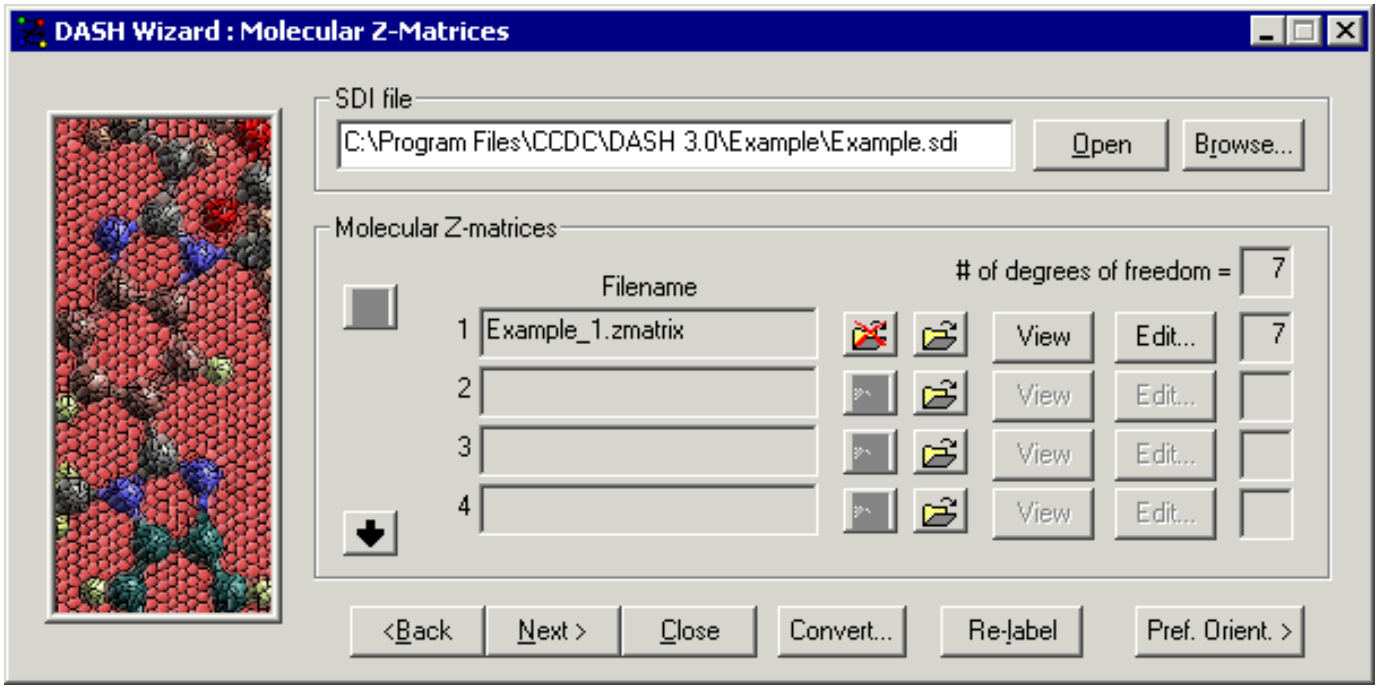
- Bond lengths
- Bond angles
- Torsion angles ranges
- Ring conformations
- Small ions
- H-bond motifs (intra- and intermolecular)

9.4 Converting Molecules to Z-Matrix Format

- Molecules built in third-party programs can be read by DASH and converted automatically into Z-matrix files (see Section 9.4.1, page 95).
- By default, the DASH will assign every single, acyclic bond as being rotatable (meaning that it will be varied during simulated annealing). This can be over-ridden, either by editing the Z-matrix file, or in DASH at the time of setting variable parameters for SA structure solution (see Section 10.3.6, page 109).

9.4.1 Using the Interface to Create Z-Matrix files

- Select **Structure Solution** either from the **Mode** menu, or by clicking the icon.
- Select a `.sdi` file from the *Molecular Z-Matrices* window that appears by clicking on the **Browse...** button.



- The allowed input formats for molecular model files are `.res, .cssr, .pdb, .mol2,` or

`.mol`.

- Click on the  icon.
- Select from displayed files (in working directory).
- Click **Open**.
- This has created a file with extension `.zmatrix` which can then be used by DASH (see Section 9.5, page 96).

9.5 Reading Molecules into DASH and Defining Rotatable Bonds

- DASH reads molecules as Z-matrices. These can be created externally, or created internally by DASH when it reads a `.mol` or `.mol2` file (see Section 9.4, page 95).
- The number of copies of a Z-matrix can be entered in the column labelled *Number*.
- DASH automatically recognises all flexible torsion angles in the molecule for non-hydrogen atoms.
- By default, the torsion angles around single, acyclic bonds will be varied through the full range of –180 to +180^{o} during simulated annealing.
- However, it is desirable to limit the number of variable parameters and their allowable ranges, since this will reduce the search space and increase the chances of structure solution. This is frequently possible with torsion angles. For example, a search of the Cambridge Structural Database shows that acyclic esters are invariably within 10^{o} of the trans-conformation. Thus, the O=C-O-C torsion angle can be constrained to a range of, say, 10 to +10^{o}, or even fixed at 0^{o}.

- Do not vary torsion angles that only affect the positions of H atoms, e.g. the torsion angles of OH, NH_2 and CH_3 groups. The data will not be sensitive to changes in these angles.
- Many other torsion constraints can be inferred from the Cambridge Structural Database.
- The best choice of constraints may depend on the quality of the data. For example, it is usually sensible to allow amides some flexibility by setting a range of –10 to +10^{o} for the central torsion angle C-N-C=O. However, if the data is poor, it is probably better to fix the torsion at exactly zero.
- It is sometimes useful to make repeated attempts at structure solution with torsion angles constrained to various likely ranges.

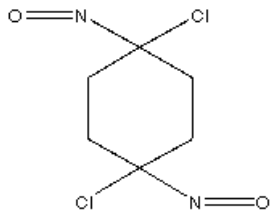
9.6 Treatment of Rings

Rings can be handled in two ways in DASH:

- You can input a likely ring conformation, obtained by looking at examples in the Cambridge Structural Database or by minimising in a modelling package, and keep the ring geometry fixed

during simulated annealing. If the structure fails to solve, you can then try an alternative ring conformation.

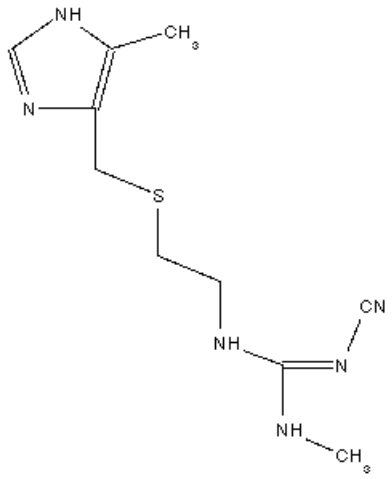
- You may have to postulate the positions of ring substituents too. For example, in the molecule below, 1,4-dichloro-1,4-dinitroso-cyclohexane, it is not only necessary to set the ring conformation (presumably chair) but also to decide whether the substituents are axial or equatorial:



- Alternatively, you can *break* one of the ring bonds and treat the resulting chain as a sequence of rotatable, acyclic torsion angles. This technique might be necessary if the ring is unusual and you have no idea about its probable conformation. However, it increases the number of variables significantly and also means that you are not taking advantage of the constraints imposed by ring closure. Thus, effectively, you are making the search space much larger.

9.7 Treatment of Stereoisomers

- If a molecule has several possible stereoisomers, you may need to try simulated annealing with each in turn. e.g. cimetidine shown here, there are possible cis or trans positions of the CN group:



- Sometimes, of course, you may be able to infer the probable stereochemistry from the chemical

synthesis or from spectroscopic evidence.

- You will not be able to determine absolute configuration from powder data.

9.8 Molecule Translation and Rotation; Special Positions

- Molecules will normally be allowed to translate and rotate freely in the unit cell, subject, of course, to the constraints of space group symmetry. This normally adds six degrees of freedom for each chemically discrete entity in the asymmetric unit, so solving structures with Z'>1 is much harder than solving structures with Z'=1.
- Rotation is expressed as quaternion numbers *Q0 – Q3*. Rotations can be restricted to a single axis (see Section 10.3.4, page 107). There are four of these, but they are not mutually independent and actually contribute only three degrees of freedom to the problem, (see Appendix H: References, page 189).
- Fixed positions are sometimes required for molecules that occupy a special position in a space group. A common example is when a centrosymmetric molecule has its centre at the origin in a centrosymmetric spacegroup. This has to be handled by introducing a dummy atom into the Z-matrix. For example, a molecule can be constrained to sit on a centre of symmetry by including a dummy atom (of any element type but with a very low site occupation factor, e.g. 0.00001). This atom is positioned on the inversion centre (0.0, 0.0, 0.0) and anchored there by clicking the *fixed* box in the translation parameter list. A bond must be input from this dummy atom to any atom in the molecule, to allow the concept of the Z-matrix to be maintained. Rotations will still be allowed for this molecule, using this atom as the molecular origin reference point.
- The easiest way to create the Z-matrix is to build a model with a model-building program, place a dummy atom at the centroid, and draw a bond to the nearest normal atom. The input this model file (mol2 or pdb format) in the normal way to the DASH Z-matrix conversion program. Then examine the Z-matrix file and edit the file to set this dummy atom to be the origin reference atom for the molecule (see Appendix G: Z-matrix format, page 185).
- There are cases where one might want to specify a certain fixed distance to be maintained between a small molecule or ion; see the example in Section 9.9, page 98.

9.9 Structures with >1 Molecule per Asymmetric Unit

If the structure contains more than one chemically-bonded unit (molecule or ion) in the asymmetric unit, each must be built separately and input to DASH. However, although you can vary the positions of more than one molecule or ion in the simulated annealing process, this has the disadvantage of significantly increasing the number of variables and so increases the complexity of the problem. Ways of avoiding this include:

- Ignore one of the molecules: if you have two molecules, one of which is relatively small and so responsible for less than (say) 10% of the scattering, (i.e. only 10% or less of the sum of all the electrons in the asymmetric unit are in the smaller molecule), then DASH may be able to find a

reasonable solution for just the larger residue. Typical examples would include leaving out water of crystallization, or an ethanol molecule in presence of a large molecule like a steroid.

- If this succeeds (i.e. produces a solution with a profile c^2 slightly higher than that expected from a complete solution with all atoms present), the resulting model can be converted into a new Z-matrix. DASH can then be instructed to use the first atom in the molecule as an anchor point, all the torsion angles being constrained to the values found in the simulated annealing run. A second structure solution can then be attempted, optimising only the rotational orientation of the main molecule and both the position and the position and orientation of the small molecule. If certain H-bonds can be assumed to be present it may be possible in fact to tether a water molecule to be at a certain distance from a donor or acceptor atom on the main molecule.
- Sometimes it is possible to guess the location of a small ion relative to a larger one. For example, in the following ion pair, it is highly likely that the chloride will be hydrogen bonded to the N-H group. Examination of the CSD database presented in IsoStar for an NH central group approached by a Cl^- ion shows an average distance of 3.1 Å.
- The method of constraining such an ion in the DASH SA procedure is best explained by this example. Using a model building program, construct a Cl atom at the required position relative to the N atom, draw a dummy-bond to the N atom, and output as a `.mol2` or `.pdb` file. On reading into the DASH Z-matrix conversion program this produces a single Z-matrix file, where the Cl atom is now tethered to the N atom. The actual distance from N to Cl can of course be modified by directly editing the Z-matrix file, as can a dummy-bond angle. In this case the torsion angle involving Cl is not meaningful and can be set as *fixed* in the parameter list.

10 STRUCTURE SOLUTION

10.1 Overview of Structure Solution

DASH solves structures by altering the positions, orientations and (where appropriate) conformations of the molecules in the unit cell, subject to the constraints of space group symmetry, until a good match is obtained between calculated and observed intensities. This process is a search for a global minimum in a multi-dimensional parameter space, the parameters being positions, rotations (expressed as quaternions) and torsion angles. This search is performed using a simulated annealing algorithm. This section covers:

- The basics of simulated annealing (see Section 10.2, page 101).
- Using the interface for structure solution (see Section 10.3, page 102).
- Simulated annealing parameters (see Section 10.5, page 117).
- Monitoring the progress of structure solution (see Section 10.8, page 124).
- Assessing the final answer (see Section 10.9, page 127).
- Troubleshooting (see Section 10.10, page 130).
- Final Rietveld refinement (see Section 10.11, page 130).
- Limitations of DASH (see Section 10.12, page 131).

10.2 Fundamentals of Simulated Annealing

- The algorithm starts by assigning random values to the parameters (molecular position, orientation and conformation).
- Agreement between calculated and observed (Pawley) intensities is assessed by computing a c^2 goodness-of-fit statistic.
- One of the parameters is randomly altered and the c^2 value recalculated.
- The algorithm accepts the new parameter value if the c^2 value has gone down (i.e. the fit has been improved). If the c^2 value has gone up, then the new value is either rejected or accepted, subject to a Boltzmann distribution. The probability of accepting such an uphill move depends on the current temperature of the system; the higher the temperature, the more likely it is that an uphill move will be accepted.
- This procedure is repeated many times, each parameter being altered in turn.
- At the end of a predetermined set of moves, the temperature is lowered, thus decreasing the chances of acceptance of uphill moves; the above process is then repeated.
- The algorithm terminates when the system converges to a minimum (hopefully, global) in c^2 space, or when a maximum number of moves is reached.

In essence, the algorithm tries to move downhill in c^2 space, but occasionally allows uphill moves in order to let the system escape from local minima.

10.3 Using the Interface for Structure Solution

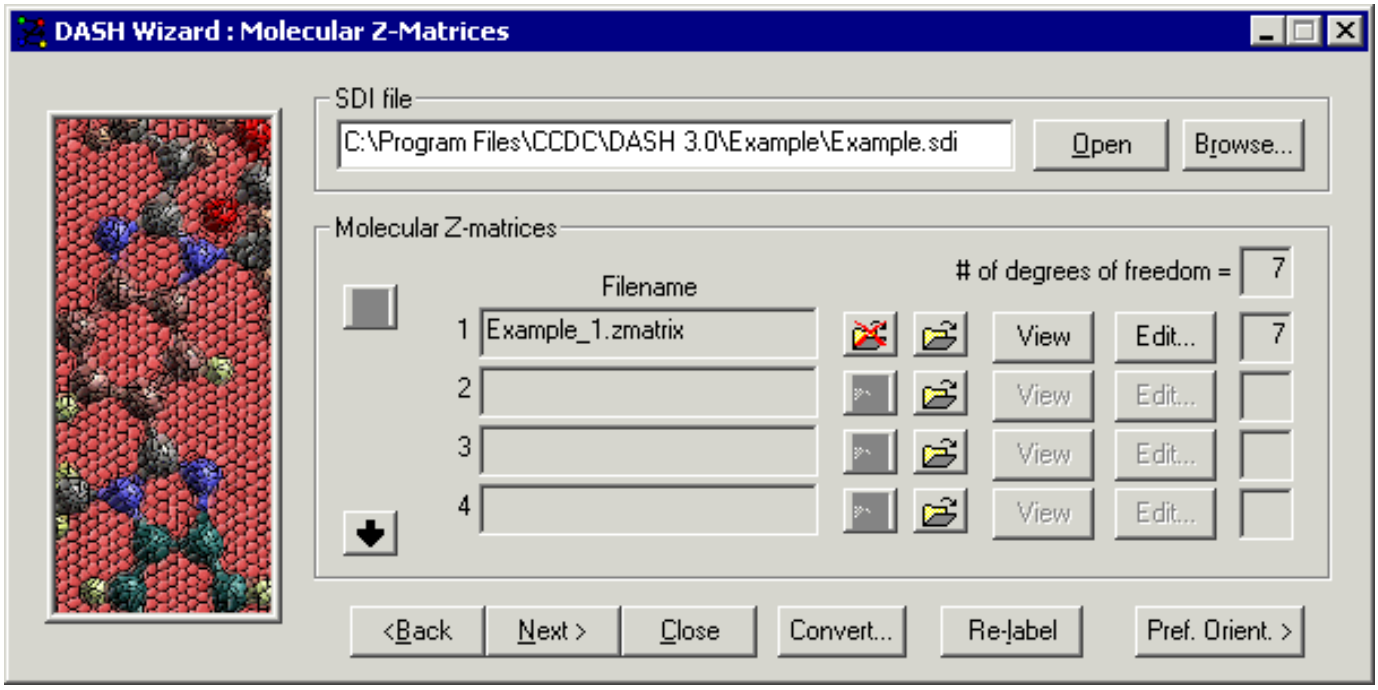
In order to use the interface for structure solution you can either:

- Call the Wizard by clicking on the following icon:

- Either select **Simulated Annealing Structure Solution**
- Or select **Structure Solution** from the top-level **Mode** menu
- Or click on the following icon:

This will bring up the *DASH Wizard : Molecular Z-Matrices* window, shown below, which you can use to:

- Input Z-matrices (see Section 10.3.1, page 103).
- Edit Z-matrices (see Section 10.3.3, page 106).
- Edit Z-matrix rotations (see Section 10.3.4, page 107).
- Look at preferred orientation (see Section 10.3.5, page 109).
- Check and set parameter ranges (see Section 10.3.6, page 109).
- Access *Mogul,* if available, via the *Parameter Bounds* window (see Section 10.4.2, page 113).
- Start the simulated annealing run (see Section 10.3.7, page 110).



- Select the Pawley-Fit file, with extension `.sdi`, saved after the Pawley refinement. Either type in the file name, or browse for files with extension `.sdi`.
- The main window now displays the diffraction data with tick marks.
- Proceed to input Z-matrix files for the molecule(s) (see Section 10.3.1, page 103).

10.3.1 Input of the Z-Matrices

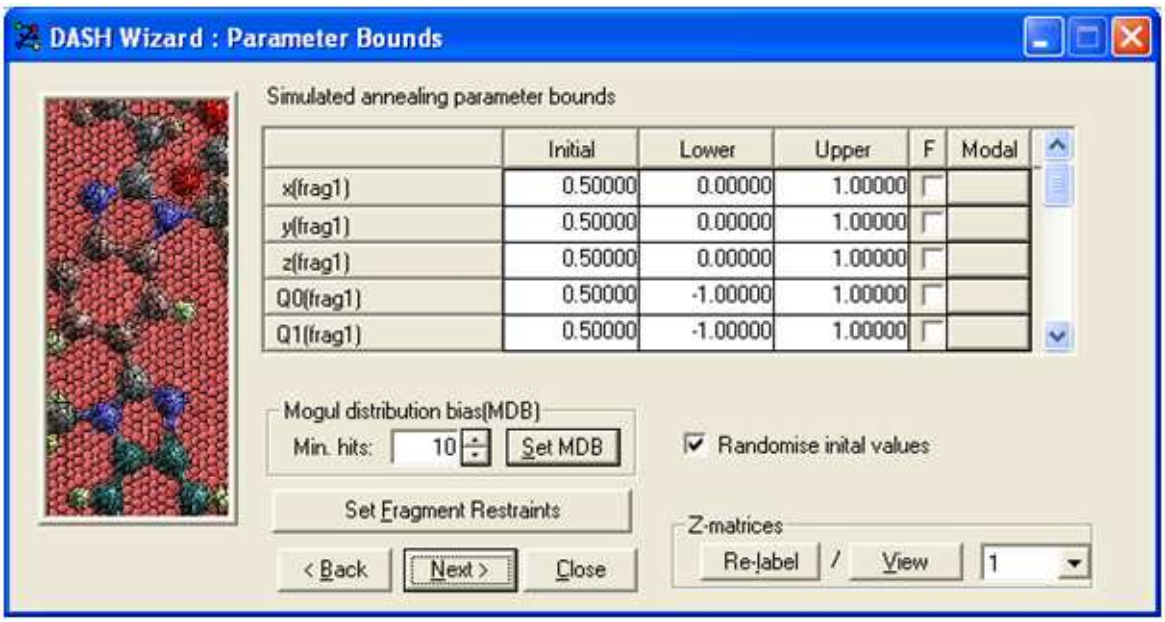
- Click on the  icon. This will display all files available with the extension `.zmatrix`, `.mol2`, `.mol`, `.ml2`, `.pdb`, `.cssr`, `.cif` or `.res`.
- Select the file and click **Open.**
- When a Z-matrix file is successfully read in the number of parameters set up is displayed to the right of the open-folder button. The first molecule should show that at least 6 parameters are created. Each rotatable torsion angle adds 1 to the total parameters.
- If there is more than one molecule in the asymmetric unit, repeat the loading process for the next Z-matrix file.
- Select **View** to view a Z-matrix. Flexible torsion angles can be colour coded by ticking the **Colour flexible torsions** check box under **Options** | **Configuration...**
- Select **Edit...** to edit a Z-matrix (see Section 10.3.3, page 106).
- Click on the  icon to clear a Z-matrix you have loaded but do not want to use.
- Then click **Next >** (see Section 10.3.6, page 109).

10.3.2 Atom tethering

- DASH features the ability to apply tethers between unassociated atoms in the solution process. The main function for atom tethering is to allow a user to break a ring in a molecule to make it flexible. A tether can then be used to make the code force ring closure while allowing the rings internal torsion angles to vary.
- The intensity c^2 is biased by the tether: Two atoms A and B are separated by a distance of d_{AB}. If the ideal distance of separation is d_{ideal} and there is a permitted tolerance of t, then the modified c^2 is given by

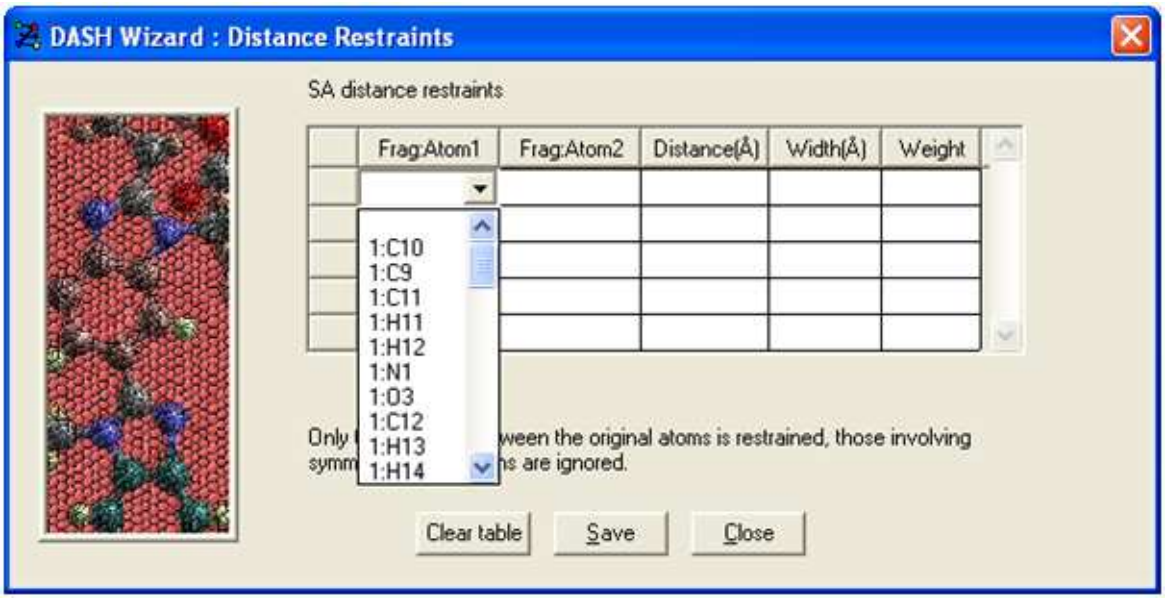
$$c^2 = c^2 + qW(MAX(0.0,[|d_{ideal} - d_{AB}| - t]))^2$$

- Where W is a user supplied weight, and q is an annealing factor: the annealing factor scales linearly from 0 to 1 as the simulated annealing progresses.
- It is also possible to tether atoms in different z-matrices to one another, to allow the user to input information regarding the presence of a given interaction.
- Atom tethering of independent z-matrices will rarely improve the success rate of the solution search for good quality data, and in certain cases may even degredate performance due to the addition of barriers into the monte carlo search process, but may be useful with poorer patterns where there is higher uncertainty in the reflection intensities.
- To use, click the **Set Fragment Restraints** in the Parameter Bounds window.

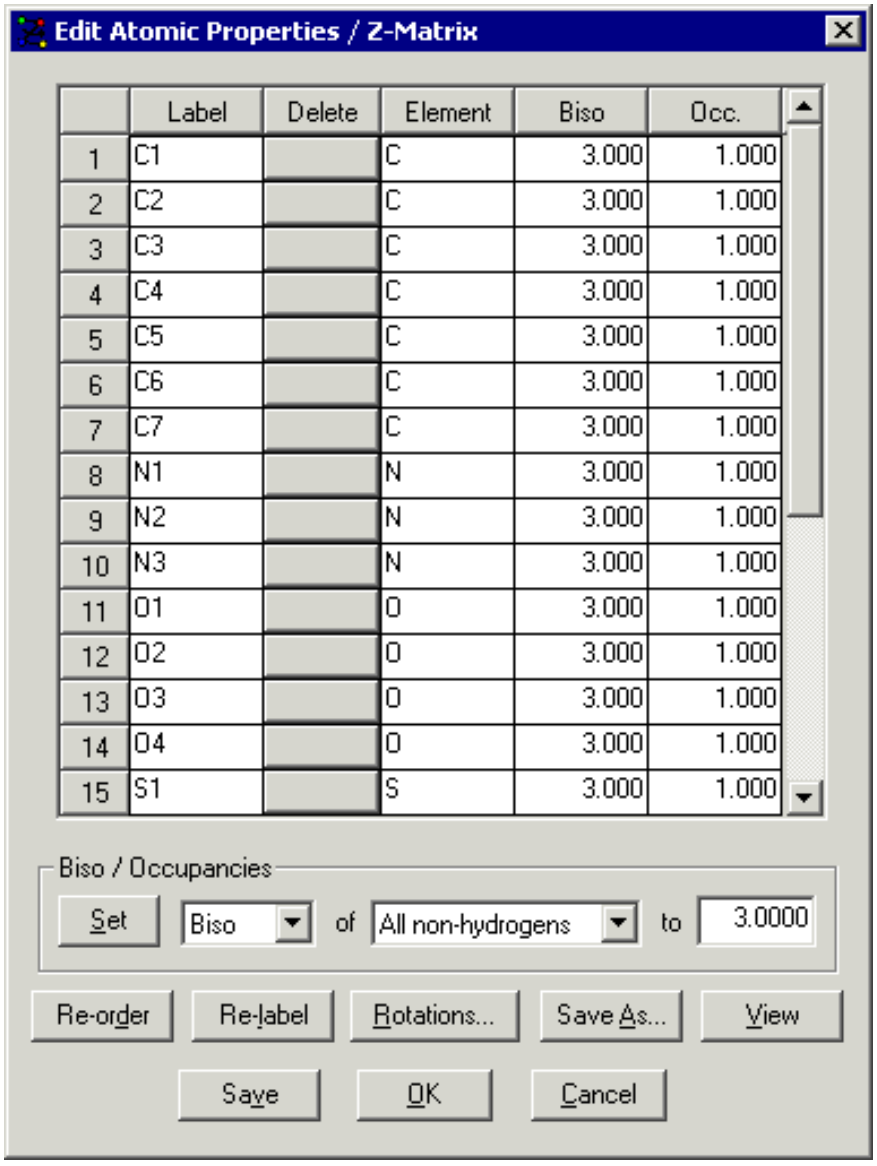


- This will launch a dialog: The user can select atoms from each fragment and a separation distance between them for 1 or more pairs of atoms, either within a single fragment or between

fragments. The user here also specifies the ideal distance between the pair of fragment atoms. A default weight is specified as 100.0.



10.3.3 Editing a Z-Matrix

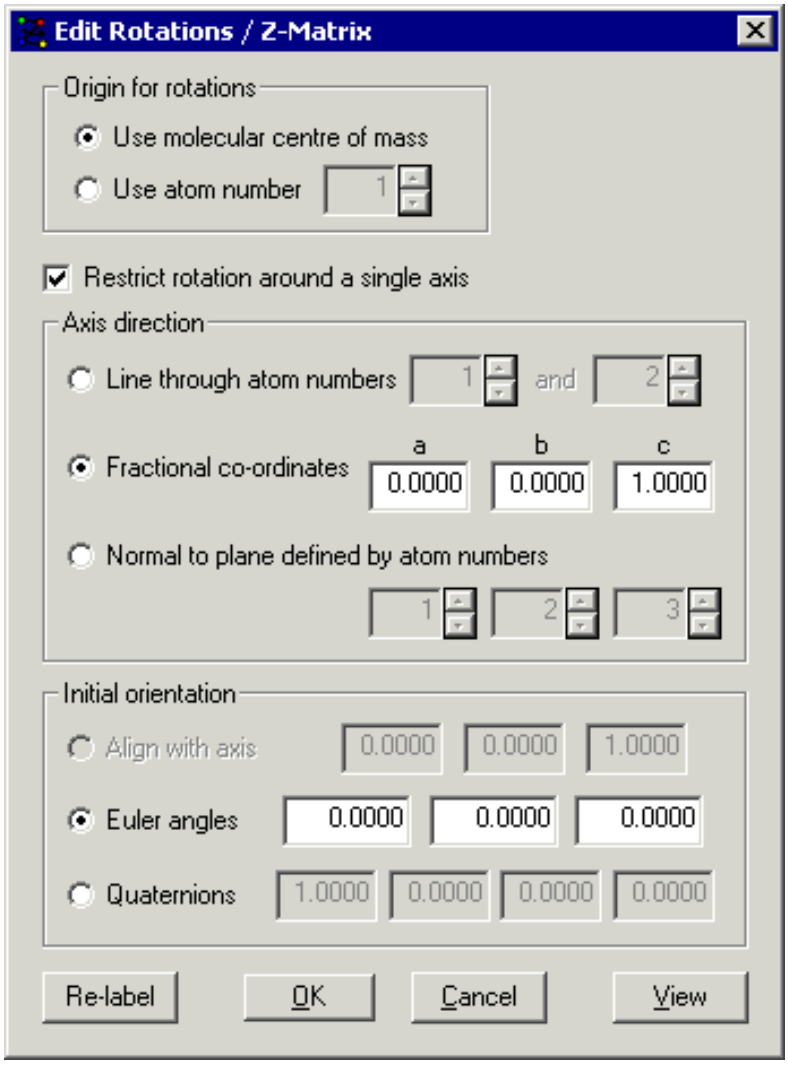


This window allows various atomic properties to be modified:

- The list of atoms displayed allows you to delete individual atoms from the Z-matrix and allows you to change the label, element, Biso and occupancy of each individual atom.
- Biso's and occupancies can be changed for groups of atoms using the **Set** button.
- **Re-order** re-orders the atoms carbon atoms first, followed by the remaining elements in alphabetical order, and hydrogen atoms last.
- **Re-label** re-labels the atoms as element + sequential number, e.g. *C7* and *Br24*.
- **Rotations...** displays the *Edit rotations / Z-matrix* dialogue box (see Section 10.3.4, page 107).
- **Save As...** allows saving of the edited Z-matrix with a new name.
- **View** displays the edited Z-matrix.

- **Save** saves the edited Z-matrix.
- **OK** returns you to the *DASH Wizard : Molecular Z-Matrices* window, keeping all changes. If atoms have been deleted, DASH will try to assemble a new Z-matrix from the remaining atoms.
- **Cancel** returns you to the *DASH Wizard : Molecular Z-Matrices* window, discarding all changes.

10.3.4 Editing Z-Matrix Rotations



This window allows full control over the way orientations of a Z-matrix are handled during simulated annealing. It is recommended that the `.sdi` file has been loaded first, so that the unit-cell parameters are available for visualisation of the centre of rotation and the initial orientation.:

- The origin of rotation can be specified in the same manner as in the `.zmatrix` file (see Section 9.8, page 98) and can be checked visually by pressing **View** and switching on the display of the cell-axes in your viewer. When using Mercury, select **Show cell axes** (lower right) rather than **Packing** (lower left).
- The rotations of molecules that lie on a rotation axis or in a mirror plane can be restricted to that

rotation axis or to the axis perpendicular to the mirror plane. When restricting rotations to a single axis, the initial orientation of the molecule with respect to the unit cell needs to be specified. This can be done by entering the Euler angles or quaternions.

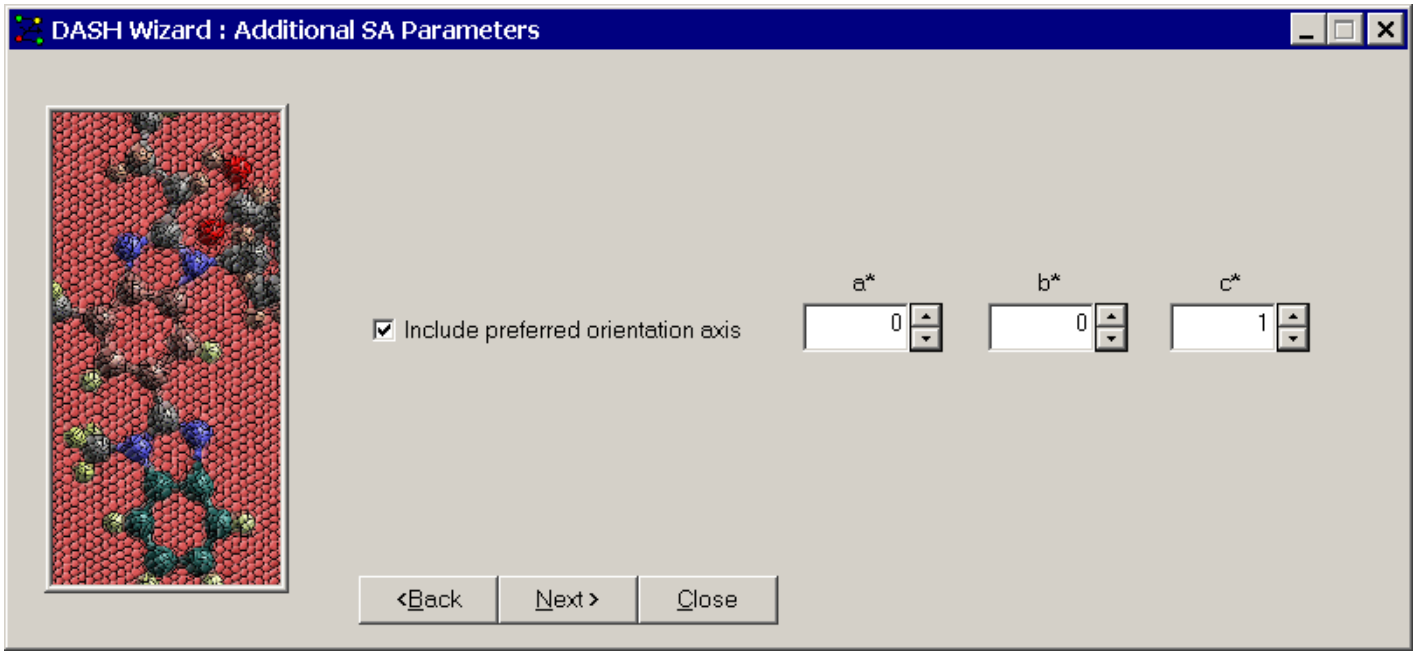
- If the axis of rotation is specified either as the line through two atoms or as the normal to the plane defined by three atoms (i.e. if the axis of rotation can be specified from the molecule alone) it is also possible to choose the initial orientation such that the axis of rotation is aligned with an axis specified in fractional coordinates. The initial orientation can be checked by pressing **View** and switching on the display of the cell-axes in your viewer. When using Mercury, select **Show cell axes** (lower right) rather than **Packing** (lower left).

The single axis of rotation is specified by the origin of rotation of the molecule and one other vector, which can be any of the following:

- The vector between two atoms. Just enter the numbers of the atoms. An easy way of finding out what the number of an atom is, is to relabel the atoms first using **Re-label** and then displaying the atom labels in Mercury.
- Fractional co-ordinates, e.g., 0,0,1 would restrict the rotation of the molecule to be parallel to the c-axis.
- Normal to a plane defined by three atoms in the molecule. Just enter the numbers of the atoms. An easy way of finding out what the number of an atom is, is to relabel the atoms first using **Re-label** in the previous screen and then displaying the atom labels in Mercury.

- **OK** returns you to the *Edit Atomic Properties / Z-Matrix* window, keeping the changes that have been made. Note that clicking **Cancel** in the *Edit Atomic Properties / Z-Matrix* window will also cancel changes made in the *Edit rotations / Z-Matrix* window.
- **Cancel** returns you to the *Edit Atomic Properties / Z-Matrix* window, discarding the changes that have been made.
- **View** displays the Z-matrix. The unit-cell axes are included, allowing you to check the origin and initial orientation of the molecule. To display the unit-cell axes when using Mercury, select **Show cell axes** (lower right) rather than **Packing** (lower left).

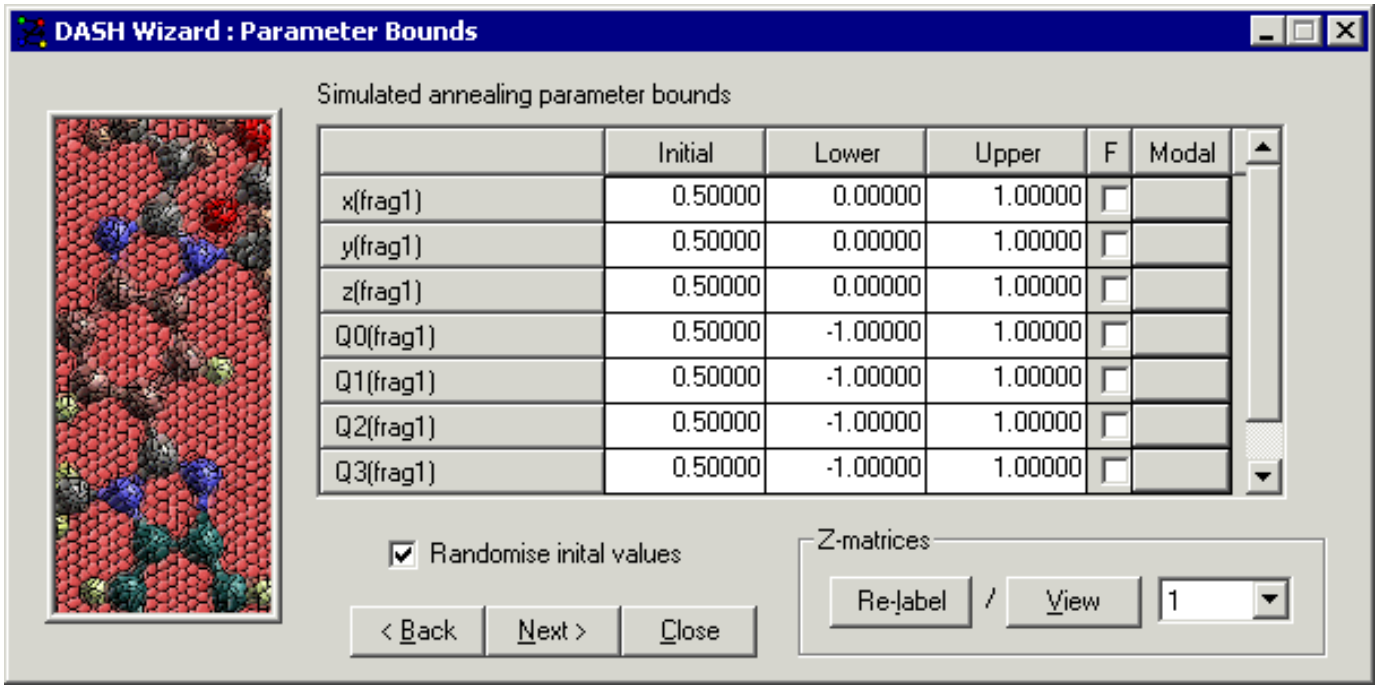
10.3.5 Preferred Orientation



The *March-Dollase* preferred orientation correction can be used. The direction of the preferred orientation must be entered in this window, the magnitude is optimised during the simulated annealing. All preferred orientation parameters are written out to all molecular output files.

10.3.6 Checking and Setting Parameter Ranges

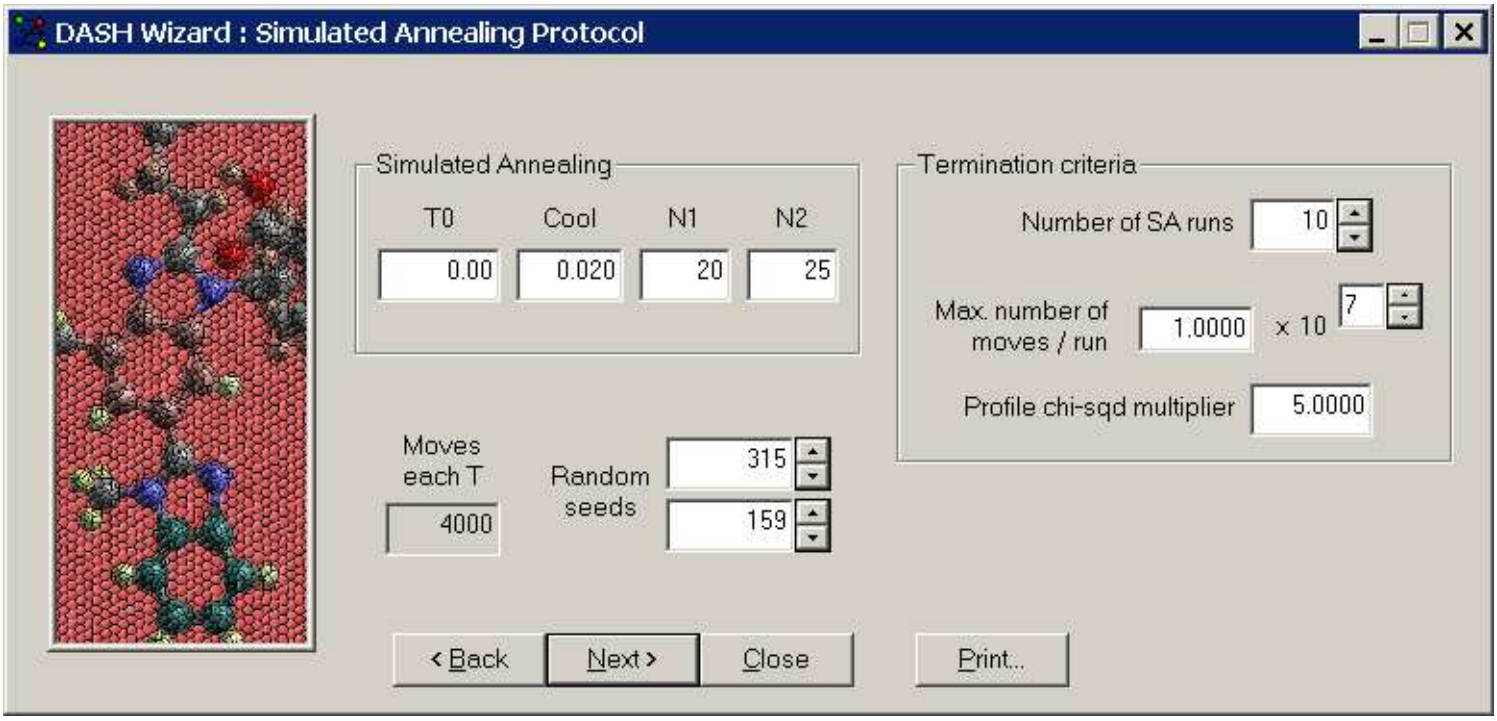
You arrive at the *DASH Wizard : Parameter Bounds* window after loading the Z-matrix files as in (see Section 10.3.1, page 103).



- The purpose of the menu is to allow you to control which parameters are variable or fixed. Each parameter has a starting value, a lower limit and an upper limit. The box **F** is ticked for fixed or unticked for variable parameter control respectively.
- If the **Randomise initial values** check box is selected, the starting values for the parameters that are varied during the simulated annealing step will be set to random values before starting the simulated annealing. When **Randomise initial values** is not selected, the values shown in this dialogue window will be used, allowing a previous run to be restarted where it left off. (If the first simulated annealing cycle is a dummy cycle to determine the initial temperature, the values will be reset at the start of the second cycle.).
- Set any of the molecular translation parameters x(Frag1), y(Frag1), z(Frag1) as fixed by clicking **F**-box if required by the space group e.g. in $P2_1$ we can fix the y-translation for the first molecule given.
- Generally it is not necessary to fix any of the molecular rotation parameters *Q0, Q1, Q2, Q3*.
- Torsion angles are not normally fixed, and will have a range of 360°. However upper and lower bounds can be entered here to define a single torsion angle range to be searched (see Section 10.4, page 111).

10.3.7 Starting the Simulated Annealing Run

You will reach the *DASH Wizard : Simulated Annealing Protocol* window from the parameter range menu (see Section 10.3.6, page 109).



This allows the user to set the variables that control the simulated annealing run.

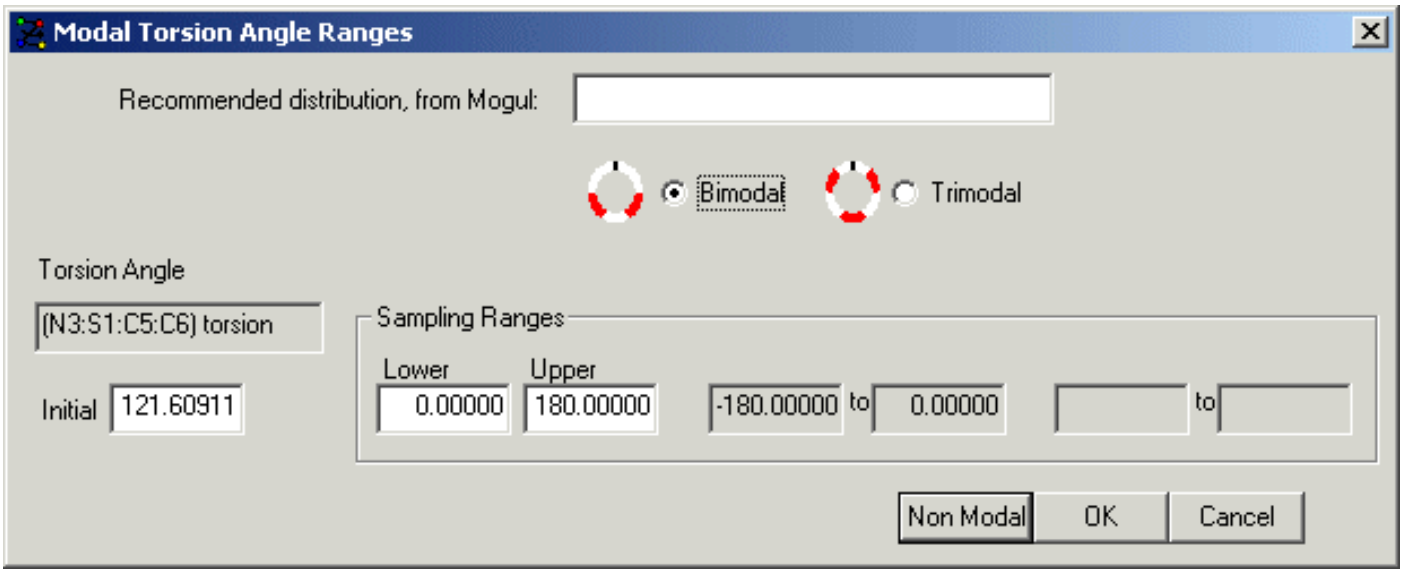
- Default values are provided which are usually satisfactory. To accept these values simply click the **Next >** button. The meaning of these parameters is discussed in Section 10.5, page 117.
- *Maximum number of SA runs*: the default setting is 10 runs. It is generally advisable to try several SA runs using different random number seeds; this is done for you automatically if you specify the number here. The best solution from each run is stored in suitably numbered files (see Section 10.9.4, page 129).
- *Maximum number of moves per run:* the default setting is 10,000,000. When this number of moves is reached, the run is terminated, whatever the value of c^2 (see Section 10.5.4, page 118).
- *Profile chi-squared multiplier*: the default setting is 5.0. This means that if the SA profile c^2 falls below a value 5.0 times that of the Pawley-fit c^2, and the minimum number of moves has been exceeded, the run is terminated. (see Section 10.5.4, page 118).
- The **Print...** button will pop up a text editor window containing a summary of the current simulated annealing parameters. This text file can be edited, saved and printed.

10.4 Setting torsion angle ranges

10.4.1 Manually entering torsion angle ranges

- Torsion angles are not normally fixed, and will have a range of 360^o. However upper and lower

bounds can be entered here to define a single torsion angle range to be searched such as -10 to 10°. If DASH has access to *Mogul*, appropriate torsion angle ranges can be explored by hitting the **Modal** button (see Section 10.4.2, page 113). Mogul is a molecular geometry database which forms part of the *CSD System* and is available separately from the *CCDC*. If Mogul is not accessible, modal torsion angle ranges can be defined in the *Modal Torsion Angle Ranges* dialogue that will appear on hitting the **Modal** button.

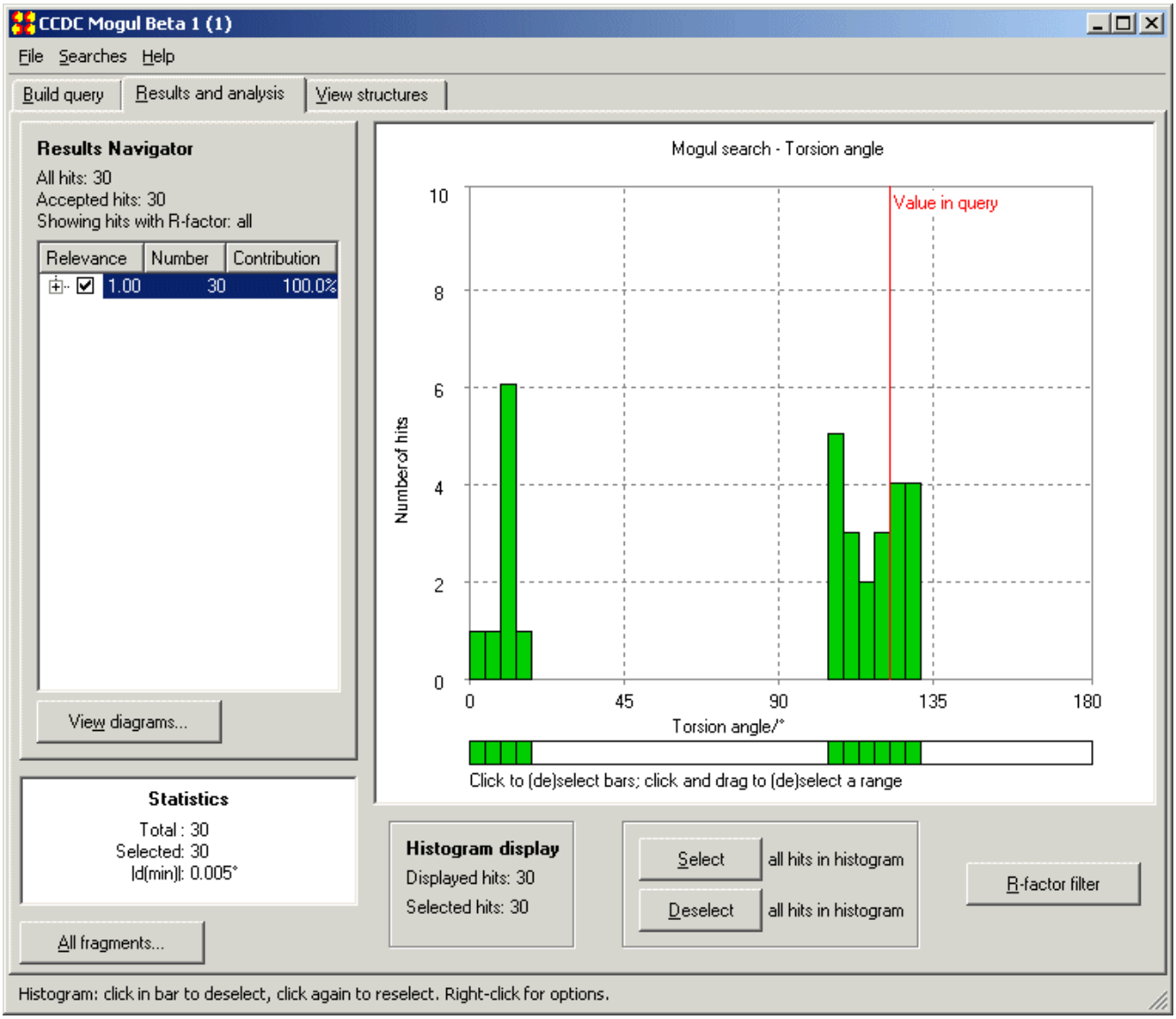


- The *Modal Torsion Angle Ranges* dialogue box allows bimodal and trimodal torsion angle ranges to be defined. The radio buttons at the top of the dialogue box are used to choose whether bimodal or trimodal torsion angle ranges are required.
- In the boxes labelled *Upper* and *Lower* enter the upper and lower bounds of a single torsion angle range, thus if a bimodal search of the torsion angle space of 30 - 90° and -30 to -90° is required, enter 30.0 in the *Lower* box and 90.0 in the *Upper* box. The complementary range is automatically determined and displayed. Torsion angles within both of the displayed ranges will be generated during the simulated annealing run. Planar torsion angle ranges (centred around 0° and 180°) can be searched by defining a bimodal range such as -20° to 20°. The complementary range determined and displayed will be -160° to 160°. If a trimodal range is chosen then torsion angles will be sampled from the defined space and two further ranges at +/- 120°.
- To accept the defined torsion angle ranges, press **OK**. This will return you to the main *Parameter Bounds* dialogue box, and the torsion angle to which modal ranges have been applied will be highlighted in red.
- Hitting **Cancel** will result in all edits, since the last accepted modal range, being ignored.
- Hitting **Non Modal** will return you to the *Parameter Bounds* dialogue box and the full 360° torsion angle range will be applied. A non-modal torsion angle range is shown in black.

- Torsion angles may be fixed at a value. Click the **F**-box, and then type in the required value in the *initial* box e.g. 0.0
- When all parameter ranges are set click **Next >** (see Section 10.3.7, page 110).

10.4.2 Using DASH with Mogul

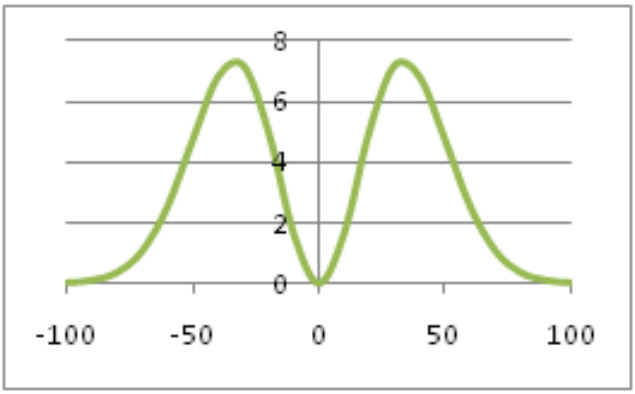
- If DASH has access to *Mogul*, appropriate torsion angle ranges can be explored using this program. Mogul is a molecular geometry database which forms part of the *CSD System* and is available separately from the *CCDC*. If Mogul has been installed in the default location, DASH should automatically find the path to the executable and this path will be displayed in the Configuration window (see Section 2.8, page 19). However, if DASH does not find the path to the Mogul executable it is possible to enter a path to Mogul in the Configuration window.
- Once a path to Mogul is present in the Configuration window, hitting the **Modal** button will launch Mogul and a histogram of the distribution of angles obtained from structures within the CSD for the chosen torsion angle will be displayed. It should be noted that the Mogul histogram displays all torsion angles, positive and negative on a positive axes, i.e. 0-180. The structures that contribute data to the histogram can be examined by clicking on the *View Structures* tab in the Mogul window.

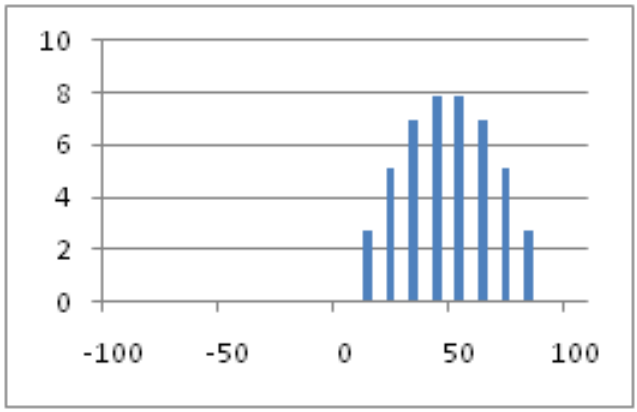


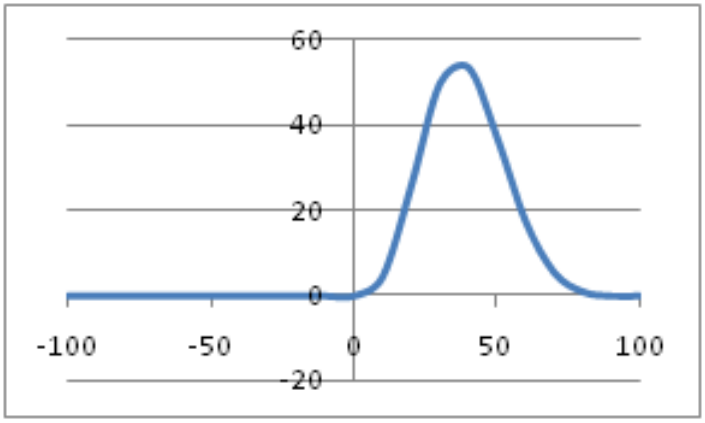
- Upon closing the Mogul window the *Modal Torsion Angle Ranges* dialogue will be displayed and, if the torsion angle had a distribution that DASH recognised, a recommended range of torsion angle values will be shown in the *Upper* and *Lower* sample range boxes. It is recommended that the user check that the suggested ranges are appropriate before accepting them. The lower and upper bounds on the ranges can be edited if different ranges are thought to be more suitable.
- To accept the defined torsion angle ranges, press **OK**. This will return you to the main *Parameter Bounds* dialogue box, and the torsion angle to which the modal ranges have been applied will be highlighted in red.
- Hitting **Cancel** will result in all edits, since the last accepted modal range, being ignored.
- Hitting the **Non Modal** button will return you to the *Parameter Bounds* dialogue box and the full 360 torsion angle range will be applied. A non-modal torsion angle range is shown in black.

10.4.3 Mogul Data Biasing

- Mogul data biasing (MDB) is an alternative way of using MOGUL to improve retrieval of correct answers in searching. MDB uses Mogul distributions to bias the sampling in searching to tend to favour regions of space where Mogul indicates there is a high likelihood.
- The underlying code works by modifying the Maxwellian distribution used for each step. In simulated annealing, each trial move is calculated by first taking a number at random from a maxwellian distribution: The maxwellian tends to favour particular moves dependent on the current annealing temperature. In MDB, the maxwellian distribution is modified to favour moves to torsion angles into regions of space that are heavily populated in the corresponding mogul distribution.
- This is summarised below. The maxwellian is binned and multiplied by the respective bin in the mogul (The mogul distributions are first modified so that no bin contains zero hits; this ensures that no parameter space is completely excluded from the maxwellian)



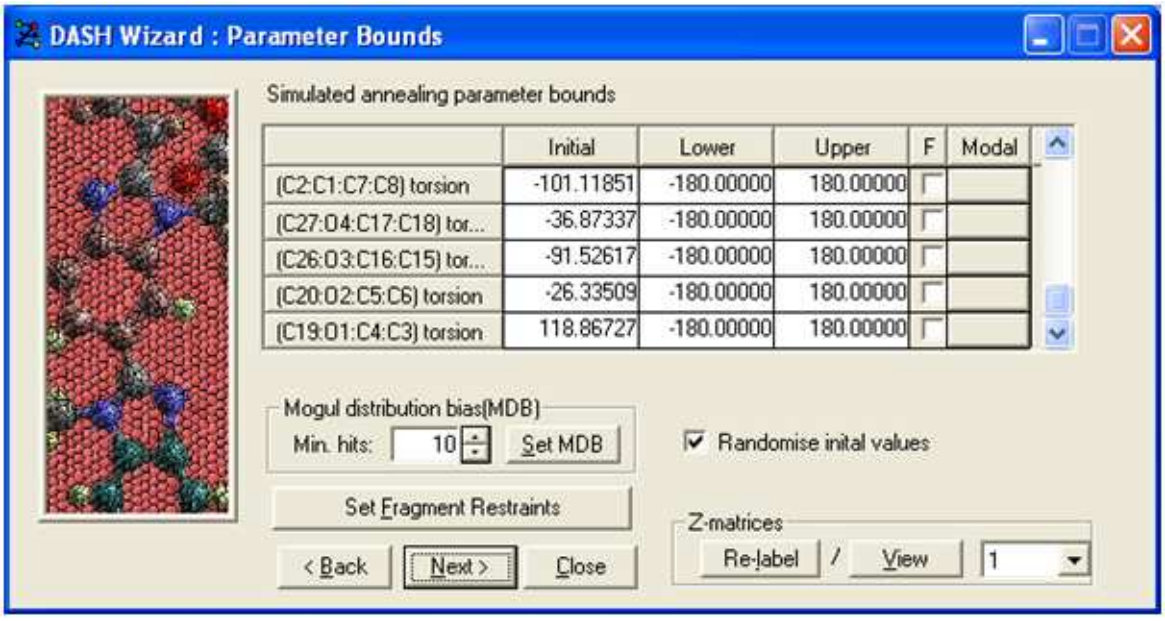




- MDB can have benefit, but on occasion can detract from the solution process. By using MDB, the user is assuming that the torsion angles in the correct solution lie within the most commonly observed ranges of torsion angles. In some structures, this clearly will not be the case; in such situations MDB will tend to bias the search algorithm away from the correct answer. Tactically, it is best to use MDB in initial runs. If, however, the structure fails to solve, still consider re-

running the solution SA without MDB as a fall back, in case your structure contains an unusual torsion angle.

- The drawbacks are mercifully rare: MDB has a beneficial effect in > 90% of the test cases that we use it for, either in speeding up the search by reaching the global minimum more quickly. In some large structures we find that MDB makes the solution process succeed in more runs than without MDB.
- MDB can be enabled by clicking the **Set MDB** button in the Parameter Bounds wizard window. The choice of distributions to use can be controlled by increasing or decreasing the minimum hits; the higher the number of hits, the more populated a mogul distribution has to be to be used in MDB. The default of 10 observations may seem a little low: Note, however, that due to the nature of the algorithm used, using distributions with low numbers of observations in fact makes little difference to the solution process, as the weight of bias to the maxwellian distribution is in proportion to the number of hits in the distribution. A distribution with only 10 observations in will not really change the sampling for a given torsion by a great amount.



- DASH also supports reading SA input settings directly from 'DBF' files. This, in principle, could allow the user to set up searches with data distributions from other sources. A given torsion angle can be biased by specifying a parameter & range for the given torsion in the input DBF file in the following form:

  -65.95955 MDB -180.00000 180.00000 18 0 0 0 0 2 7 10 5 0 0 0 0 0 0 0 0 2 14

  Here, the parameter's start value is first, followed by the control keyword 'MDB'. Subsequently we have the maximum & minimum value range for the parameter followed by the number of bins in the associated histogram. Finally we have the histogram itself. Clearly the histogram

could easily be altered to reflect, say, the range of torsion angles observed in a conformational analysis for the associated molecule.

10.5 Simulated Annealing Parameters

10.5.1 Starting Temperature

- If the search space is complex (i.e. contains many local minima), the starting temperature must be high so that the algorithm will make many uphill moves in the early stages. This will ensure that the search space is explored thoroughly.
- Setting *T0* to zero in the interface instructs DASH to select the starting temperature automatically. This is recommended, at least in the first simulated annealing run, until you get a *feel* for the problem.
- DASH selects the starting temperature by performing a brief simulated annealing run at high temperature and monitoring the variance of the c^2 value as random moves are made in parameter space. Based on the results, the starting temperature is set to a value that will allow the algorithm to escape deep local minima at the outset of the search (i.e. the higher the variance in c^2, the higher the starting temperature).
- The optimum starting temperature is very data dependent; it could be 25 K in a good case (a simple response surface with few local minima), but much higher (>1000 K) for a complex problem.

10.5.2 Cooling Rate

- DASH typically uses a fixed, conservative cooling rate of 0.02 K (i.e. the rate at which the temperature is reduced) for the annealing process. The temperature reduction is applied at the end of each cycle of annealing.
- The cooling rate is not constant as the annealing proceeds. If DASH detects large fluctuations in c^2 (implying that the algorithm is in an interesting region of parameter space) it automatically reduces the cooling rate to ensure a thorough search.
- The slower the cooling rate, the more thorough the search of parameter space and the greater the chances of finding the global minimum. However, a slow cool obviously takes longer.

10.5.3 Number of Moves

The values of *N1* and *N2* determine the number of moves (random parameter changes) that are made at each temperature. Specifically, if there are N variable parameters (positional, rotational, conformational), the simulated annealing performs *N1*N2*N* moves at each temperature. Default values are *N1* = 20 and *N2* = 25; these will only need to be increased in difficult cases.

10.5.4 Convergence Criteria

How does DASH know when it has a correct answer, and thus when to stop the annealing process? The c^2 obtained from the Pawley fit represents very much the best fit that can be obtained from the data (all intensities are treated as being variables in the least-squares process) and so if DASH comes close to achieving this profile c^2 in the annealing process, there is a good chance that the answer is correct.

There are a number of reasons as to why you will not obtain as good a fit in the structure solution process as you did with the Pawley fit:

- Your input model is based on a number of chemical assumptions, some of which may not be entirely accurate.
- the assumption is that all non-H atoms have fixed temperature factors.
- There may be some preferred orientation in the sample.

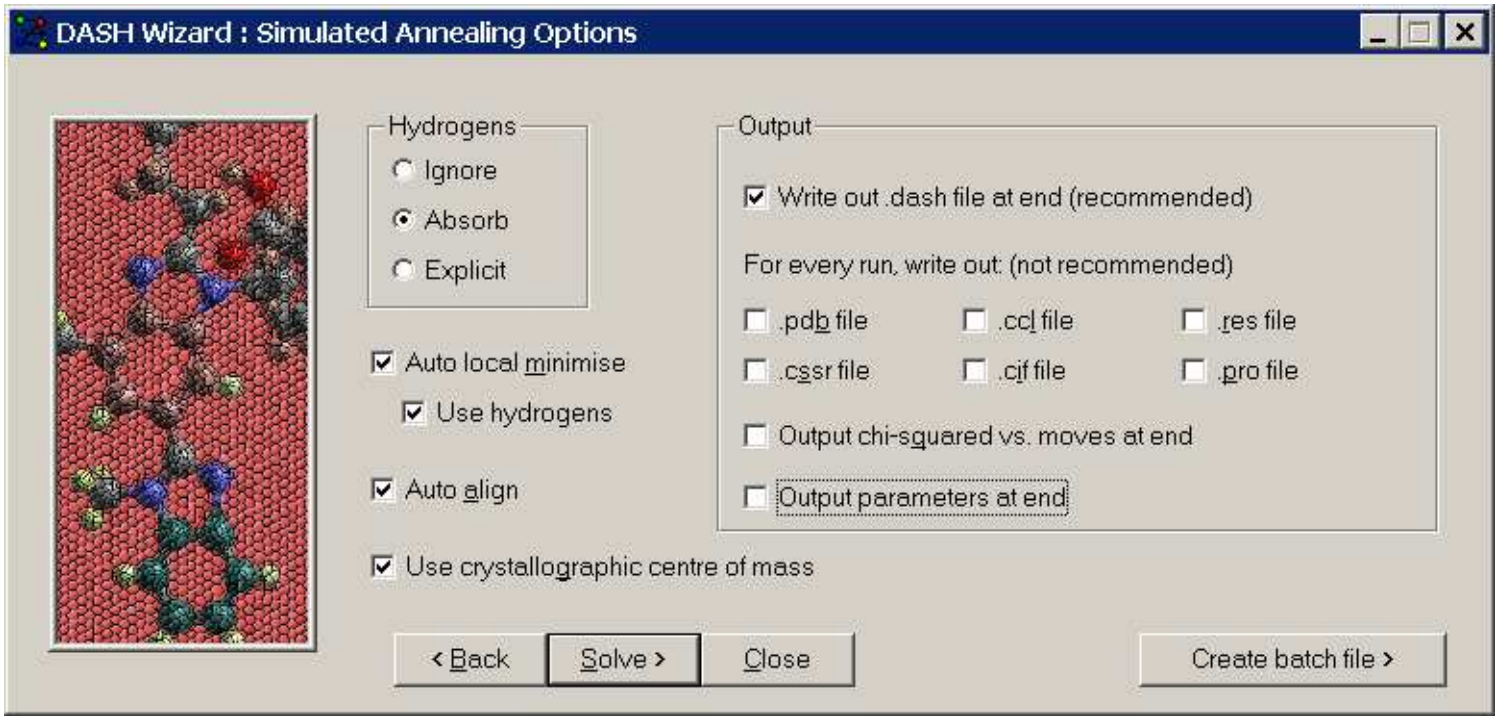
Accordingly, DASH realises that if the profile c^2 comes within a preset multiple (default value = 5.0) of the Pawley c^2, then there is a good probability that this structure is worth examining. For example, with the default multiplier setting, if you achieved a Pawley c^2 of 3.7, then the SA process would terminate when the SA profile c^2 fell below a value of 18.5. The multiplier setting is user controllable via a field on the SA control panel.

There is always a chance that the SA process may become trapped in a local minimum with a profile c^2 value above the pre-set cut-off. In such circumstances, the SA could in principle, run forever. For that reason, there is a pre-set maximum of 10,000,000 SA moves in DASH. The majority of structures will solve well before this number of moves is reached! You can reduce the maximum number of moves if required, but note that there is a pre-set minimum of 1000 moves.

10.5.5 Random Number Seeds

- Simulated annealing is a random process that depends on computer-generated random numbers. Random-number generators use a set of *seeds* which determine the sequence of random numbers used within the program. Changing the seeds will change the sequence and thus alter the route taken by the algorithm through c^2 space.
- Different seeds used for otherwise identical runs will generate different paths. Conversely, keeping the same set of seeds between otherwise identical runs will result in identical paths. This can be useful in demonstrating situations, as a set of random seeds that produces an answer quickly can be noted and used again.
- Note that when you ask for multiple runs, DASH automatically calculates a new set of random seeds for each run.

10.6 Simulated Annealing Options



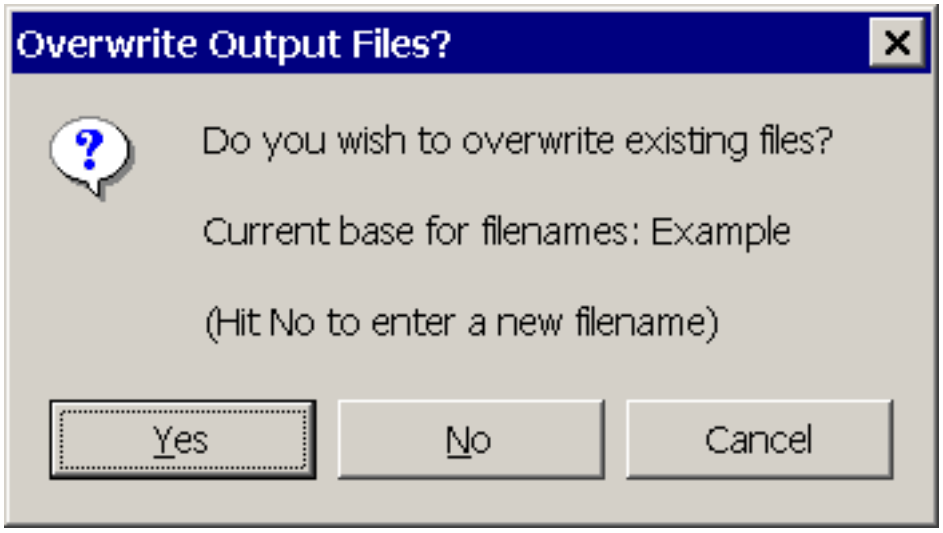
- **Hydrogens**: The **Absorb** option (default) takes account of the electron density of hydrogen atoms by increasing the occupancy value of their riding atom by the appropriate amount. Alternatively, since the scattering power of hydrogen atoms is low, contributions from hydrogens can be ignored. If you wish to take account of the hydrogen positions directly, then the **Explicit** option can be used.
- **Auto Local Minimise**: when selected, the c^2 of each final solution is minimised using a simplex algorithm before the solution is written out. If **Use Hydrogens** is selected, hydrogens are included in the local minimisations of the final solutions.
- **Auto Align**: when selected the molecules of the final solution are aligned before the solution is written out.
- **Use crystallographic centre of mass**: when selected each atom is assigned a weight of Z^{-2} when the molecular centre of rotation is calculated, where Z is its number of electrons. Otherwise, no weights are applied.
- **Create batch file**: Click this button to write files that can be submitted to a grid, or to write files that can be used to run DASH in batch mode.(see Section 11.2, page 133)

Options for saving files are as follows:

- **Write out .dash file at end**: enables you to save all solutions plus the diffraction pattern and the Pawley fit in one binary file with the extension `.dash`. This file is written once the simulated

annealing run is complete. The `.dash` files can be reopened within DASH to view solutions obtained from previous runs of DASH.(see Section 10.9.4, page 129)

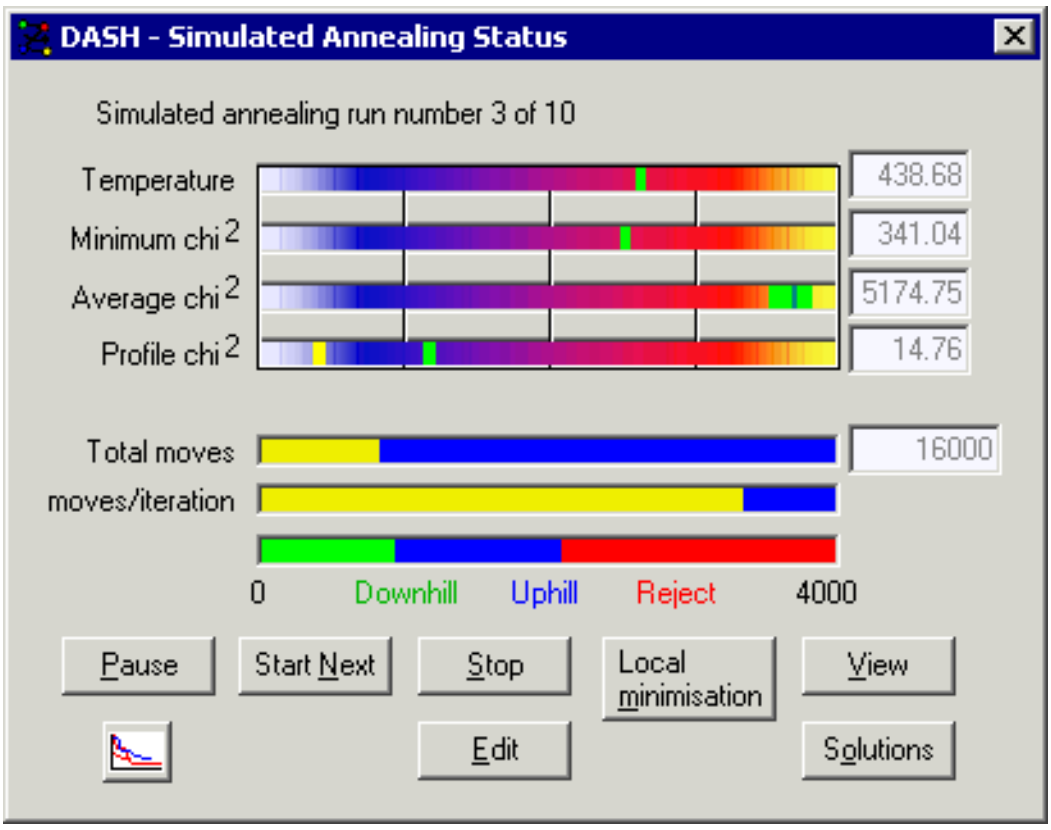
- You may also choose to write out a file at the end of each simulated annealing run that contains the coordinates of the best solution obtained, i.e. the solution with the lowest c^2 value. This file will be given a default name using the Pawley-fit File name selected, e.g. *sucrose55.sdi* will produce a file *sucrose55.pdb* for example. The structure files also contain the intensity and profile c^2 values of the solution. Options for file formats are:
  - **.pdb**: Additional information contained in this file includes the DASH simulated annealing parameters as well as the translations, Q-rotations and torsion angles.
  - **.cssr**: a file is written in `.cssr` format.
  - **.ccl**: a file is written in `.ccl` format
  - **.cif**: a file is written in `.cif` format
  - **.res**: a file is written our in `.res` format
  - **pro**: when selected a file with the extension. `.pro` is written out which contains 2q, the observed profile, the calculated profile for the best solution and the original esds. The file is written out in ASCII format and can be imported into a spreadsheet package such as Excel
- **Output Chi-squared vs. moves at end**: when selected a graph of the profile c^2 versus moves is written out to a file in ASCII format with the extension `.chi`, at the end of the simulated annealing, This can be imported into a spreadsheet package such as Excel.
- **Output parameters at end**: when selected a.tbl file is written at the end of the simulated annealing run: this file contains the translations, the Q-rotations and the torsion angles of all solutions. This can be exported into a spreadsheet package such as Excel.
- If you wish to keep the results for several runs started with different values of the parameter-ranges (see Section 10.3.6, page 109), or simulated annealing protocol (see Section 10.3.7, page 110), you are given the option to input a new name for each run. This occurs in a popup menu immediately on the first display of the *Simulated Annealing Status* window (see Section 10.7, page 121).



- For example you might want to do separate runs keeping torsion angles 3 or 4 fixed, and give file names like this: *sucrose.tor3fixed*, *sucrose.tor4fixed*, etc., which will output best solution files: *sucrose.tor3fixed.pdb*, *sucrose.tor4fixed.pdb*, etc.

10.7 Status Display of Simulated Annealing Run

You will reach this menu after clicking **Solve** >in the *Simulated Annealing Protocol* window (see Section 10.3.7, page 110). It enables you to monitor the progress of the Simulated Annealing Run.



Status information:

- Simulated annealing run number: in the above example current run 3 of a set of 10.
- *Temperature*: the current SA temperature value.
- *Minimum chi*^{2}: the minimum c^2 for the integrated intensities i.e. the quantity that is being minimised by the SA.
- *Average chi*^{2}: the average value of the minimum c^2 for the integrated intensities.
- *Profile chi*^{2}: the c^2 for the diffraction profile, and it is directly comparable with the c^2 for the Pawley fit.
- *Total moves*: the total number of moves in the SA run so far.
- *Moves/iteration*: the total number of moves performed (NS times NT times number of parameters) before a temperature reduction is applied.
- *Downhill/Uphill/Reject*: the number of downhill/uphill/rejected moves in last iteration of 4000 moves.

Buttons:

- Pausing the Simulated Annealing Run

  The **Pause** button simply pauses the DASH program to free up processor time for some other purpose. Click **OK** to continue with DASH.

- Starting the next Simulated Annealing Run

  When in a multi-run, the **Start next** button terminates the current run and starts the next.

- Stopping the Simulated Annealing Run

  The **Stop** button stops the simulated annealing run immediately and advances to the *DASH Wizard : Analyse Solutions* window (see Section 10.9.4, page 129).

- Editing the Simulated Annealing Parameters

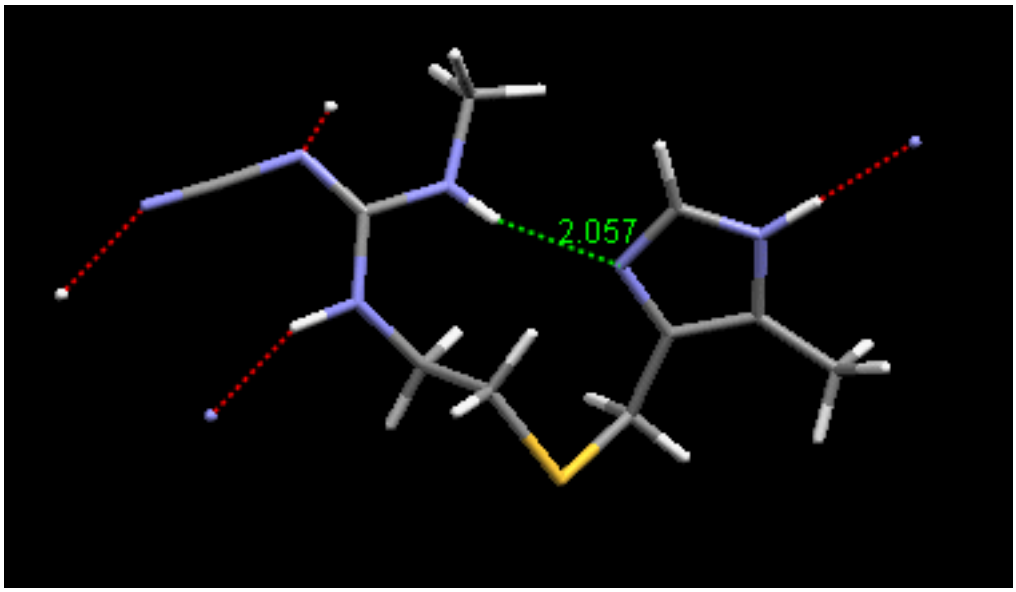
  The **Edit** button stops the simulated annealing run immediately and returns you to the *DASH Wizard : Parameter Bounds* window (see Section 10.3.6, page 109)

- Simplex Optimisation of the Best Solution
  - This **Local minimisation** button pauses the run, and takes the parameter values for the best

solution to date as a starting point for as Simplex minimisation (see Appendix H: References, page 189). A pop-up window appears giving the improved value of the c^2 for the integrated intensities, which can be compared with the minimum c^2 in the *Simulated Annealing Status* window. You can then continue from this improved position by clicking **Yes**, or ignore this by clicking **No**.

- Since the DASH implementation of simulated annealing varies one parameter at a time in the random path, this local minimisation point can have a dramatic effect in speeding up the final stage of the process of finding the lowest c^2 point. However it will have no useful effect until the search has reached a point reasonably near to the global minimum. Therefore, it is generally used once a good fit to the data has been achieved, in order to quickly take the structure to the best minimum in the vicinity of the current structure.

- Viewing the 3D Structure of the Best Solution
  - As the simulated annealing run proceeds DASH keeps a record of the best solution found to date. The **View** button opens up the default 3D-Visualiser immediately for the current version of this best solution.
  - The CCDC visualiser program Mercury is supplied with DASH, an example image is shown here of the best solution found for cimetidine (see Tutorial example 4). It is easy to display hydrogen bonds for quick checking and here it can be seen that all expected donors and acceptors are satisfied. By clicking on the ends of H-bonds one can see the connected molecules and assess the H-bond networks pattern. It is important here also to check quickly for any impossibly close contacts between atoms.



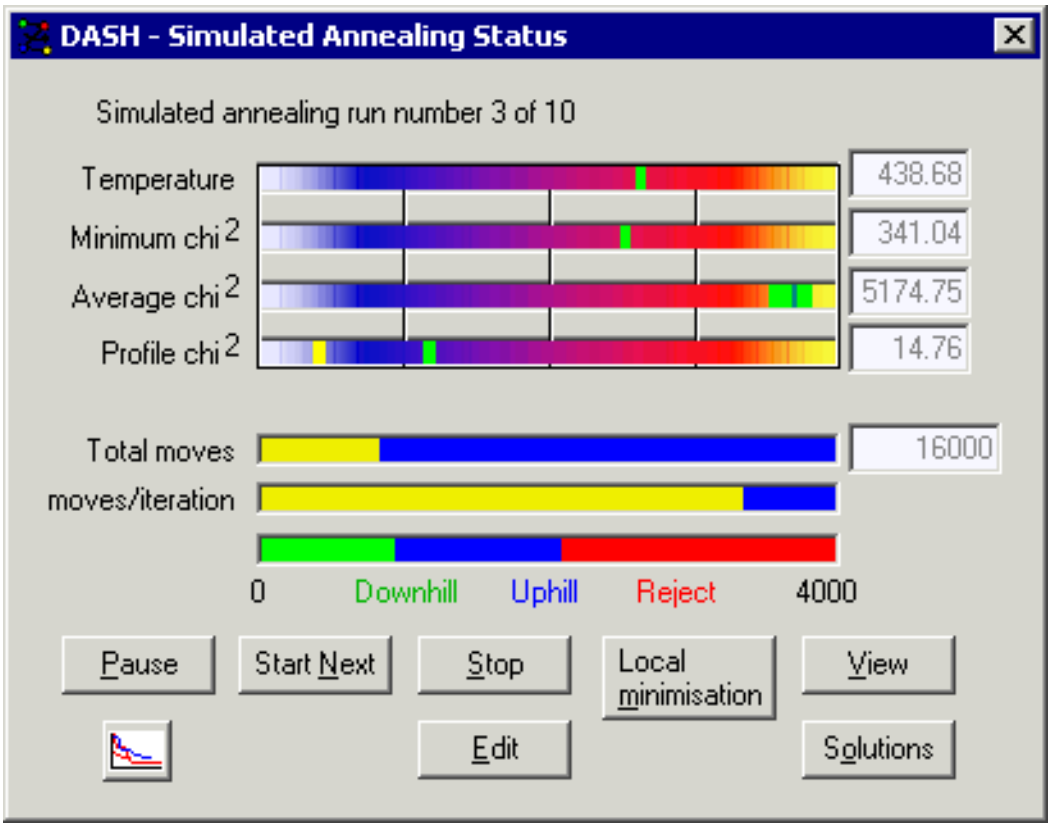
  - The 3D visualiser is not automatically kept up-to-date with the best solution. In order to look at the latest solution, you must click **View** again.

- The choice of visualiser is controlled via **Configuration...** which can be selected from the **Options** menu bar.

10.8 Monitoring the Progress of Structure Solution

As a simulated annealing run proceeds, DASH displays a number of diagnostic statistics, the most important of which are:

- Various c^2 statistics (see Section 10.8.1, page 124).
- The current temperature, the total number of moves made so far, the number of downhill moves, and the number of uphill moves that are accepted or rejected (see Section 10.8.2, page 125)
- Whether or not to stop a Simulated Annealing run (see Section 10.8.3, page 125).
- Monitoring the progress of structure solution (see Section 10.8.4, page 126).



10.8.1 Interpreting c^2 Statistics

DASH displays the minimum and average values of the correlated integrated-intensities c^2 and the best value of the profile c^2 for the structural model. The Pawley fit c^2 can be viewed by selecting **Pawley / SA** from the **View** menu.

- The *minimum correlated integrated-intensities* c^2 is the quantity that is actually being minimised. This is the minimum value obtained so far of the c^2 statistic that measures the fit between the reflection intensities calculated from the structural model and the intensities extracted from the Pawley fit.
- The *average* c^2 is the average value of the correlated integrated-intensity c^2 at the current temperature.
- The *profile* c^2 for the structural model measures the fit between the powder profile calculated from the best structural model so far and that observed experimentally. This c^2 value is on the same scale as the Pawley fit c^2, which makes it the most useful statistic for assessing how close the structure solution is to the best fit possible.
- If the profile c^2 for the structural model is well above the c^2 obtained from the Pawley fit, the structure is some distance from the true crystal structure.
- If the profile c^2 for the structural model is close to the Pawley c^2 (within a factor of 2-5) the structure is probably solved.
- Note however, that sometimes the profile c^2 may be up to 10 times the value of the Pawley c^2, and the structure is still basically correct. The exact ratio depends upon many factors, such as accuracy of the input model and extent of preferred orientation.

10.8.2 Interpreting Current Temperature and Number of Moves

- A great many moves (perhaps several million) may be needed for the structure solution of a large flexible molecule, and it is not unreasonable to leave DASH running overnight.
- Ideally, you should get about an equal proportion of uphill, downhill and rejected steps early on in the annealing.
- If the starting temperature was too low, you will find that the number of accepted uphill moves is small, even though the profile c^2 for the structural model is still much higher than the Pawley c^2. This means that the search is trapped in a local minimum and you should stop the run and restart at a higher starting temperature.

10.8.3 Deciding Whether to Stop a Simulated Annealing run

The Pawley fit c^2 can be viewed by selecting **Pawley / SA** from the **View** menu.

- Use of the **Local minimisation** button at any time during structure solution activates a simplex minimisation from the current best position. This allows you search the area around the current minimum in order to assess the best attainable c^2 in the vicinity of the current well (i.e. the minimum in search space that is currently being explored). You should normally only invoke this search if the current profile c^2 is within a factor of 5-6 times the value of the Pawley c^2 and

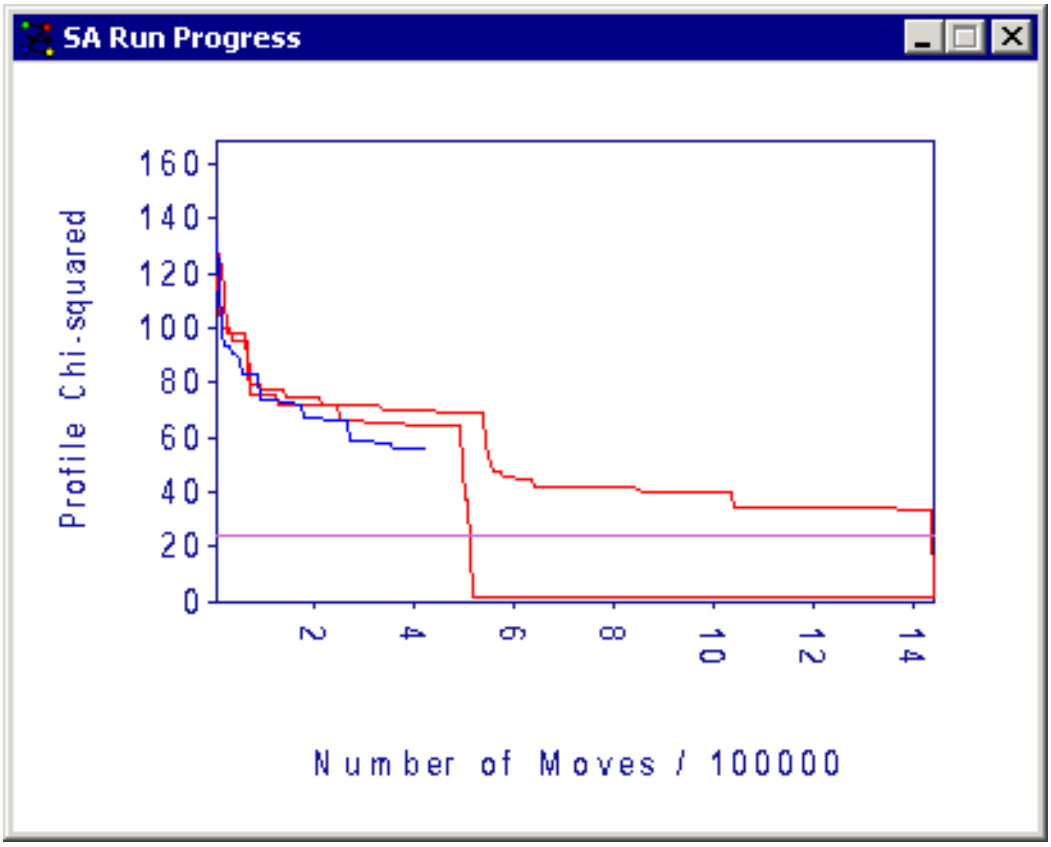
you want to accelerate the final stages of the search. If the simplex search brings the intensity c^2 down significantly (NB: the simplex warning box currently reports the Intensity c^2, not the profile c^2) then the structure will have been pulled in very close to the final answer, so you can accept the results of the simplex and stop the annealing. If not, just hit **No** and allow the annealing to continue from the position it occupied prior to the simplex.

- DASH can automatically test for early termination. Early termination testing tries to speed up searching by attempting speculative local minimisation of DASH runs when a given run's profile c^2 gets to within a threshold of the fitted pawley c^2. The user specifies an early termination criterion in the simulated annealing. If the SA gets within twice of this threshold, then new good solutions are minimised; if the minimised structure has a profile c^2 less than the specified termination criterion the solution is accepted and the SA run is terminated.
- If the c^2 is a large multiple of the Pawley c^2 (say, a factor of 10 or higher) and a reasonable proportion, say 10%, of uphill moves are still being accepted, then the annealing has not converged and it should therefore be left running.
- If the number of rejected uphill moves is about 50% of the total moves being made, and the profile c^2 is still very high, then the search is probably trapped in a local minimum. You should stop it and start again at a higher starting temperature.

- Note that DASH can spend a long time apparently *stuck* at one c^2 value, but remember that it is continually sampling parameter space, so as long as uphill moves are being accepted at a decent rate, be patient!
- For very large molecules it may be necessary to leave the program running overnight to achieve solutions.

10.8.4 Monitoring the Progress of Structure Solution

A graph of Profile c^2 vs. Number of Moves is plotted as the simulated annealing runs progress. The end point of the simulated annealing run, the product of the Pawley c^2 and the multiplier chosen in the *Simulated Annealing Protocol* window is shown on the graph as a horizontal line. The graph can be zoomed in on using the left mouse button and the Home key resets the view to full scale.



10.9 Assessing the Solution

Important questions to ask are has the data been fitted and, subsequently, does the structure make sense. You therefore need to examine:

- The profile c^2 (see Section 10.9.1, page 127).
- The visual match between calculated and observed profiles (see Section 10.9.2, page 128).
- The crystal packing (see Section 10.9.3, page 128).
- The files saved from multiple simulated annealing runs (see Section 10.9.4, page 129).
- Reproducibility of solution (see Section 10.9.5, page 130).

10.9.1 Assessing the Final Profile c^2

- The first thing to look at is the profile c^2 for the structural model. As a rough guide a good solution should have a profile c^2 of around 2-3 times the value of the Pawley c^2. Accurate models will give small multiples of the Pawley c^2, but if your starting model is not particularly accurate, you may still get the correct answer but with a much higher multiple.
- If the profile c^2 for the structural model is much more than 10 times the Pawley c^2, then the

structure is almost certainly wrong, even though there may be elements of truth in it.

- The c^2 value can be forced up by a variety of effects:
  - Your data may have a systematic problem (e.g. preferred orientation, K_{a2} contributions for laboratory data).
  - You may not be modelling all of the scattering in the unit cell (e.g. there might be a solvent, or perhaps the compound is not exactly what you think it is).
  - You may have truncated the data at the wrong point.
  - You may have fixed parts of the molecule in the wrong conformation.
  - Your estimates of particular bond lengths / angles may be significantly in error.

10.9.2 Visual Comparison of Observed and Calculated Profiles

- Visually compare the observed and calculated profiles. Are there any peaks that are very poorly fitted? For example a strong peak for which there is little or no calculated intensity? This is a warning sign, although it could always be due to an impurity, an instrument spike, or preferred orientation.
- At high angle, there may be a reasonable fit to the data but an overall mismatch of intensities due to an incorrect overall isotropic temperature factor. Systematic forfeiting of the high angle data implies that the temperatures factors for the atoms have been set too low, whilst underfitting implies that the temperature factors, B, are too high. The DASH default values of B = 3.0 for non-hydrogen atoms and B = 6.0 for hydrogen atoms are normally sufficiently close to allow structure solution. These defaults can always be altered in the input Z-matrix.

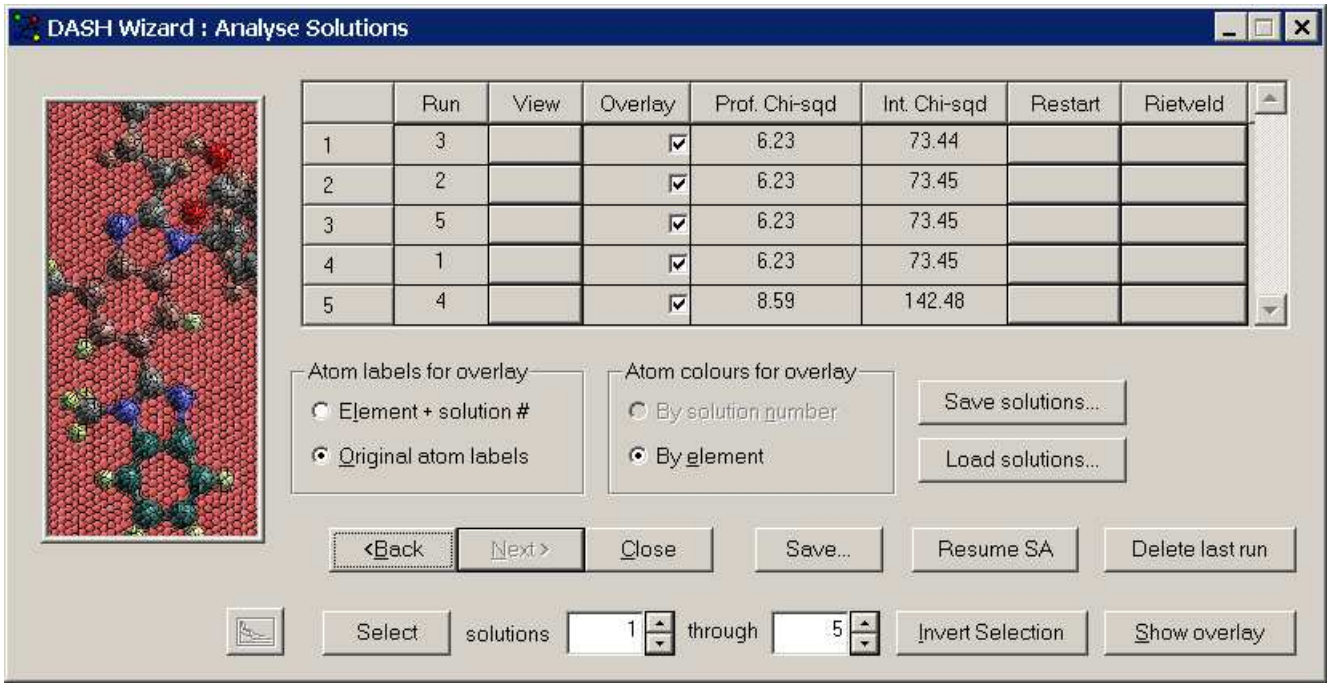
10.9.3 Inspecting the Crystal Packing

An important indicator of whether a solution is correct is the network of interactions that are formed in the structure:

- Check that there are no unreasonably close intermolecular contacts in the crystal. An occasional, marginally short contact (e.g. an H…H distance of just under 2 Å) may be expected, given that the structure is effectively of low resolution, but anything more extreme indicates that the solution is at least partially wrong.
- Large void spaces are equally unlikely, unless you suspect the presence of, e.g., solvent which has been left out of the calculation.
- Check that likely interactions are formed: for example, it is extremely rare for an N-H or O-H group not to be involved in a hydrogen bond. Remember that, in the majority of cases, hydrogen atoms will have been placed in assumed positions and will not be subject to adjustment, other than as a consequence of adjustments made to the main backbone of the molecule. Hence, an O-H group that does not appear to H-bond might well do so if the H atom is rotated to a new position.
- Finally, remember to check the molecular conformation: does it compare well with similar molecules in the Cambridge Structural Database?

10.9.4 Files saved from multiple runs of SA

If multiple runs of the SA procedure have been requested (see Section 10.6, page 119), the program will keep a record for each run of the lowest c^2 found, and the name of the corresponding solution co-ordinate file. The solution files names are created from the given name of the run with the text suffix _001, _002, … The solutions are sorted into ascending order of **Profile Chi-squared** in the display:



- Selecting the **View** button for a specific solution allows you to view the crystal structure and the calculated profile. The overlay check boxes allow you to select the solutions for which the crystal structures are to be shown overlaid in a single unit cell.

- Any of the simulated annealing solutions can be Rietveld refined by clicking the appropriate **Rietveld** button. Usually, this will only be done for the best solution.
- **Save...** allows you to save the solutions as `.pdb, .cssr, .ccl, .cif, .res` and `.pro`. You can also save the final values of the SA parameters and the c^2 progress.
- **Save solutions...** enables you to save all solutions plus the diffraction pattern and the Pawley fit in one binary file with the extension `.dash`.
- **Load solutions...** enables you to load solutions with the extension `.dash`.
- **Resume SA** allows you to resume the simulated annealing where it left off. New runs will be appended to the existing one.
- **Delete last run**: enables you to delete the last solution listed in the summary window. A `.dash` file saved after solutions have been deleted will not include the solutions deleted.
- Selecting a solution's **Restart** button starts the simulated annealing with this solution's parameters as the initial parameters. The initial parameters are not randomised and existing runs are overwritten.

10.9.5 Reproducibility of Solution

A good indicator is the reproducibility of the structure solution using different starting seeds. If the process keeps converging to the same minimum after multiple simulated annealing runs, you can have increasing confidence in the solution.

10.10 Things to Try When Structure Solution Fails

- Review the original data; e.g. do the esds look reasonable; have all corrections been applied?
- Review the indexing and the Pawley fit; if necessary, try solving the structure in a different space group.
- Check the molecular stereochemistry, or ring conformation, or whatever else might be relevant. Might there be solvent present and are you sure of the molecular structure?
- Have you frozen any torsion angles around single bonds? If so, consider releasing them.
- Conversely, if the number of parameters is large, try reducing the search space by fixing torsion angles to likely values or, at least, to smaller ranges.
- Try altering the non-hydrogen atom temperature factors.
- Persevere: if the data are reasonably good, Z'=1, and the number of rotatable torsions is < 10, DASH should be able to solve the structure with ease.

10.11 Final Rietveld Refinement

- The solution found by DASH may be verified by Rietveld refinement using a program such as TOPAS, GSAS, FullProf or RIETAN. DASH provides an interface to TOPAS, GSAS and RIETAN.

- It is also possible to do a rigid-body Rietveld refinement in DASH (see Section 12.1, page 137).
- Initially, try refining only the scale factor and a global temperature factor.
- Following this, if necessary, you can attempt to use highly constrained Rietveld refinement of the atomic positions and isotropic temperature factor and/or refine preferred orientation.
- You should consider using all the available data for the Rietveld refinement.

There are a great many packages capable of performing Rietveld refinement of an output structure. Many of these are commercial and are shipped with diffractometers so consult your diffractometer manufacturer for details. There are also several freely available packages, such as DBWS, CCSL, GSAS, and FullProf. A good starting point is to visit *http://www.ccp14.ac.uk*,where many of the programs can be downloaded, together with examples and tutorials.

10.12 DASH Limitations

- Generally, given reasonable data, DASH is routinely successful on structures containing one molecule in the asymmetric unit and up to 10 flexible torsion angles. With more flexible molecules, or with structures containing two molecules in the asymmetric unit, the solution is often found if care is taken.
- Hard limits: 300 atoms, 600 reflections, 15000 data points.
- Although DASH is at the heart of the structure solution process, it is not completely stand-alone. You will need to use some other programs, all of which can be obtained easily and with little or no expense. Specifically, you will need programs for:
  - Checking for possible unit cells of higher symmetry (see Appendix A: Programs for Indexing and Cell Reduction, page 157).
  - Building molecules in 3D (see Appendix B: Programs for Building 3D Molecules, page 157).

11 RUNNING DASH IN GRID MODE OR BATCH MODE

11.1 Overview of DASH in Batch/GRID Mode

Structure solution can now be performed by DASH using distributed processing to speed up the procedure. This can be achieved in a number of ways depending on the resources at your disposal whether this consists of a single-processor computer, a multi-core machine, or an entire network of computers. The use of distributed computing can be particularly helpful when individual SA runs are long due to a complex problem.

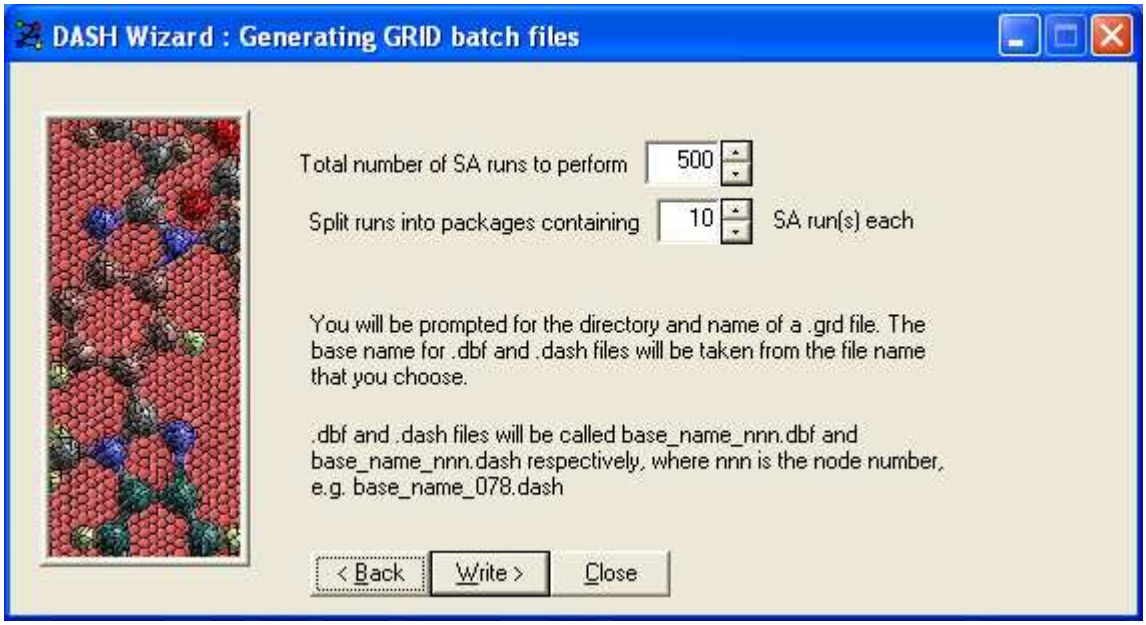
This section covers the following:

- Setting up the input files (see Section 11.2, page 133))
- Using DASH in batch mode (see Section 11.3, page 134)
- Using DASH in GRID mode (see Section 11.4, page 135)
- Post-processing of the results (see Section 11.5, page 135)

11.2 Setting up Input Files

During the process of setting up a series of SA structure solution runs there is the option of creating batch files (see Section 10.6, page 119): clicking on the **Create Batch File** button will access the *Generating GRID batch files* dialogue box. This section of the wizard will allow you to create the input files necessary to run DASH in a distributed fashion.

The first step is to choose the total number of SA runs that are required; these runs are then split into packages with a user-defined number of runs in each. If, for example, the user chooses 500 SA runs, split into packages of 10 SA runs each, this will produce 50 packages. Each package is represented by a `.dbf` file which gives DASH the instructions for that set of SA runs. A `.grd` file is also created which lists the filenames for each of the separate packages. For running DASH across a GRID, it is recommended to use packages containing just one SA run each, this allows greater flexibility when assigning packages to different nodes of the network.



The `.dbf` files that are created store all of the parameters defined for the SA runs, so if a particular option is chosen in the *Simulated Annealing Options* window then it will be adhered to in batch mode. Note: The `.dbf` files are in simple text format and can be edited using a basic text-editor such as Notepad or Wordpad in order to, for example, modify one parameter then re-run a set of SA runs.

To generate the input files, click on the **Write >** button. At this point you will be prompted for the output directory and the filename for the `.grd` file. The `.dbf` files and the `.dash` files (when the jobs are finished) will be named using this filename as the base along with a number corresponding to the particular SA run package.

11.3 Using DASH in Batch Mode

11.3.1 Drag-and-Drop Running

Individual `.dbf` files can be dropped on to the DASH executable or the shortcut icon on your desktop. In this mode DASH will run in the background without any displayed user interface. Multiple SA packages can be run concurrently in this manner but no progress or final results will be shown. The output files will be sent to the directory where the input files were residing (see Section 11.5, page 135)).

11.3.2 Multi-Core Processing with MDASH

For computers with multi-core processors there is also a currently unsupported tool called MDASH which allows the controlled distribution of sets of SA runs across the system. The tool provides an interface displaying the progress of the SA runs and will pop-up the DASH program to show the results when completed. For further information on MDASH see the MDASH documentation within

the DASH 3.1\Unsupported Extras directory.

11.4 Using DASH in GRID Mode

For users with access to a GRID-enabled network of computers, DASH can also be run in GRID-mode. To achieve this it is necessary to set up the client PCs and the GRID servers for distributed DASH. This application of DASH is not currently fully supported, but installers to set up the computers to use DASH across the GRID are available from the DASH page of the CCDC website (*http://www.ccdc.cam.ac.uk/products/powder_diffraction/dash/*) along with further documentation.

11.5 Post-Processing of Results

After running DASH in batch mode or in GRID mode there will usually be a large number of .dash results files output. In order to process these results it is generally easier to combine all of the files into one large .dash file. There are two ways of doing this:

- Open a command prompt and navigate to your results directory, then type "C:\Program Files\CCDC\DASH 3.1\DASH.exe MERGE D:\Results Directory\ output.dash". This will automatically merge all the .dash files in the directory (D:\Results Directory) to produce the file output.dash.
- Open a command prompt and navigate to your results directory, then type "C:\Program Files\CCDC\DASH 3.1\DASH.exe MERGE". A window will then appear which will allow you to navigate to your results directory and give the name for your merged dash file.

12 RIGID-BODY RIETVELD REFINEMENT.

12.1 Rietveld Refinement in DASH

Rietveld refinement is a technique for minimising the differences between the modelled crystal data and the experimental powder pattern. This means that it is a very useful method for validating DASH solutions and for producing publication-quality crystal structures.

There are currently a large number of different programs available for performing Rietveld refinement. Within DASH there is a built-in rigid-body refinement module and interfaces to three external programs; TOPAS, GSAS and RIETAN. These interfaces are designed to facilitate the transfer of structural data to the external program and allow an inexperienced user to carry out a basic structural refinement. Each of the interfaces will not necessarily provide the best route through a refinement for any specific structure, but are intended to offer a starting point for using the particular program.

No support is provided for the external programs, which are supported to differing extents by their own developers. The interfaces from DASH to each program, along with the built-in rigid-body refinement module, will be supported.

Web-links for external programs:

- TOPAS - *http://www.bruker-axs.de/index.php?id=topas*
- GSAS - *http://www.ccp14.ac.uk/solution/gsas/*
- RIETAN - *http://homepage.mac.com/fujioizumi/rietan/angle_dispersive/angle_dispersive.html*

12.1.1 Options for Rietveld Refinement in DASH

There are currently four available options for Rietveld refinement in DASH.

Firstly, there is a built-in module for performing rigid-body Rietveld refinement which uses the rigid bodies (Z-matrices) from the simulated annealing stage. The use of rigid bodies imposes a large number of constraints on the atomic coordinates, which makes it more likely that the Rietveld refinement will result in a chemically reasonable crystal structure. Another advantage of this approach is that it is not necessary to include the atomic resolution data, so that the background and peak shape parameters from the Pawley refinement can be used.

Rietveld refinement can also be carried out in a semi-automated way using the DASH interface to one of three external programs; TOPAS, GSAS and RIETAN. These three interfaces prepare the reflection data for the external programs, set up the refinement input files and facilitate refinement in a series of pre-defined steps in order to carry out a basic structural refinement.

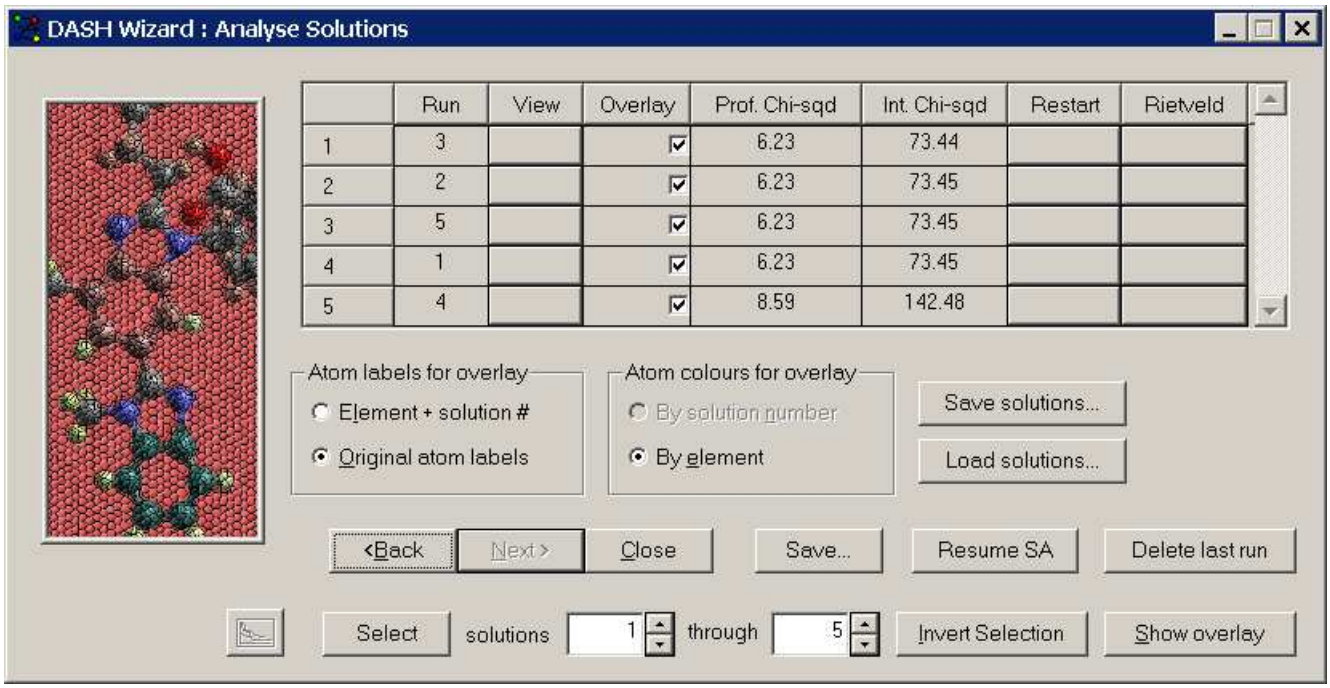
The interface between DASH and the refinement packages will only become available once the relevant sections of the Configuration Window (Choose Options-> Configuration from the main toolbar) in DASH have been completed.(see Section 2.8, page 19)

12.1.2 Starting Rietveld Refinement

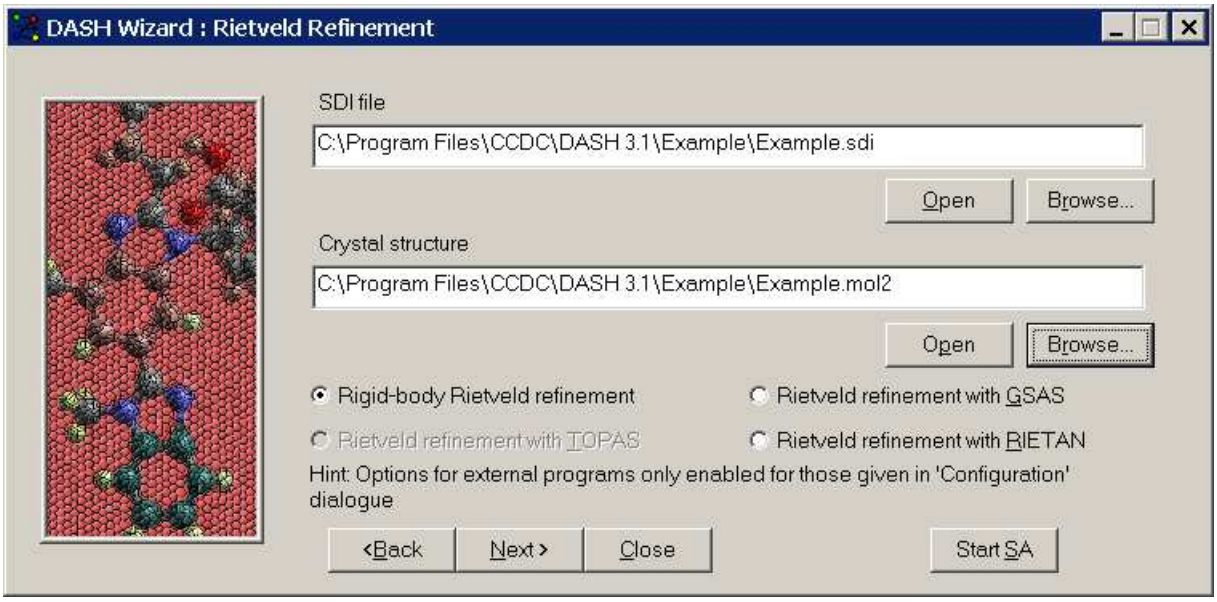
There are two ways to start the Rietveld refinement:

- using the solution from a simulated annealing run.
- with the crystal structure from e.g. a `.cif` or a `.res` file.

The first option is recommended. Note that it is possible to save all solutions from the simulated annealing and to read them back in at some other time (see Section 10.9.4, page 129).



In order to do a Rietveld refinement for a crystal structure that is not a simulated annealing structure from DASH, choose **Rietveld refinement** in the main Wizard window. The following window will appear:

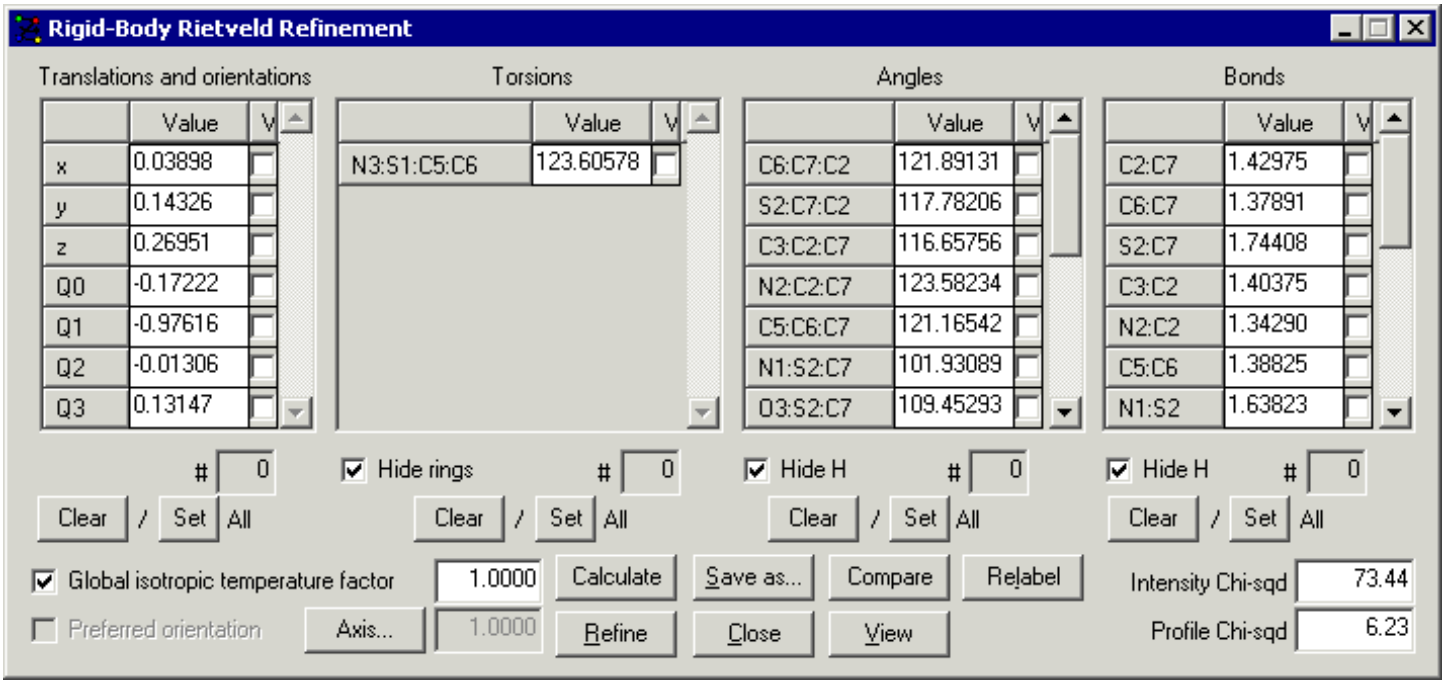


It is also possible at this point to start an SA structure solution run using the given crystal structure coordinates, without randomisation of initial coordinates, by clicking on the **Start SA** button.
Note that there can be ambiguities with respect to unit-cell settings and space-group settings: DASH only knows one setting for $P2_12_12_1$, and loading a crystal structure file with a different space group setting will produce erroneous results.

12.2 Rigid Body Refinement

12.2.1 Rigid Body Refinement Dialogue Box

The *Rigid-Body Rietveld Refinement* dialogue box can be accessed from the *Analyse Solutions* dialogue box after a simulated annealing run:



Refinable parameters are:

- positions and orientations of all Z-matrices
- torsion angles
- valence angles
- bond lengths
- global isotropic temperature pre-factor
- preferred orientation (if used during simulated annealing)

In order to refine a variable, its check box must be selected. The check boxes for positions/ orientations, torsion angles, valence angles and bond lengths are labelled *V* for variable and can be switched on and off either individually or as a group by using the **Clear** and **Set** buttons.

Due to the low x-ray scattering power of Hydrogen, torsion angles, valence angles and bond lengths involving one or more Hydrogen atoms are hidden by default and not refined.

The global isotropic temperature pre-factor is a factor that pre-multiplies the isotropic temperature factors of the individual atoms. This allows different atoms (e.g., different elements) to have different isotropic temperature factors.

Buttons:
Calculate: calculates the powder pattern with the current parameters without refinement.
Save as... allows you to save the current crystal structure to a `.pdb, .res, .cssr, .ccl` or `.cif` file, and allows you to save the current powder pattern (both experimental +

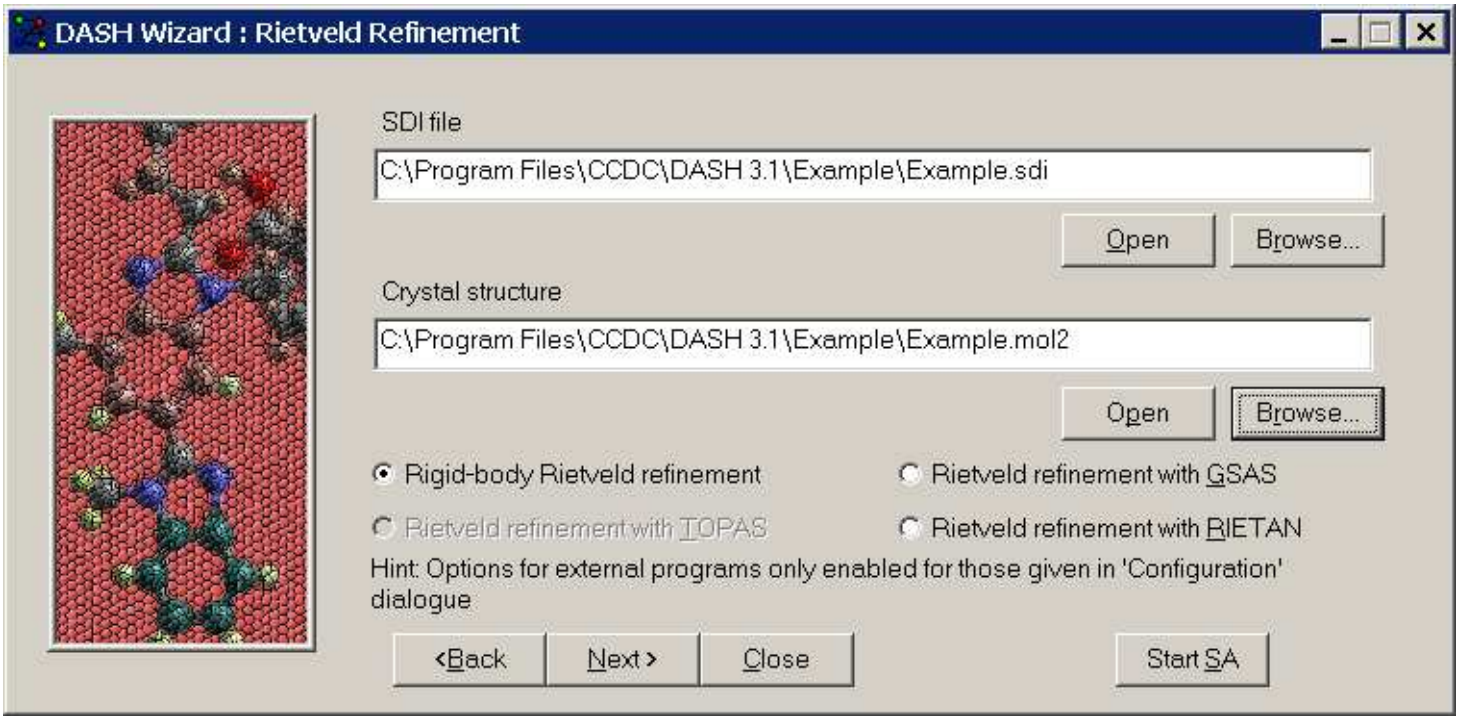
calculated) to a `.pro` file.
Compare: displays the original crystal structure and the Rietveld refined crystal structure superimposed.
Refine: performs a Rietveld refinement.
Close: closes the window.
View: displays the Rietveld refined crystal structure.
Axis... allows you to specify an axis to include the March-Dollase preferred orientation model.
Relabel: pressing **Relabel** relabels all atoms in all Z-matrices. This is useful for identifying e.g. torsion angles if a molecular model used for building a Z-matrix did not have unique atom labels.

Note that it is possible to manually enter values for all refinable variables. These values will be effective immediately.

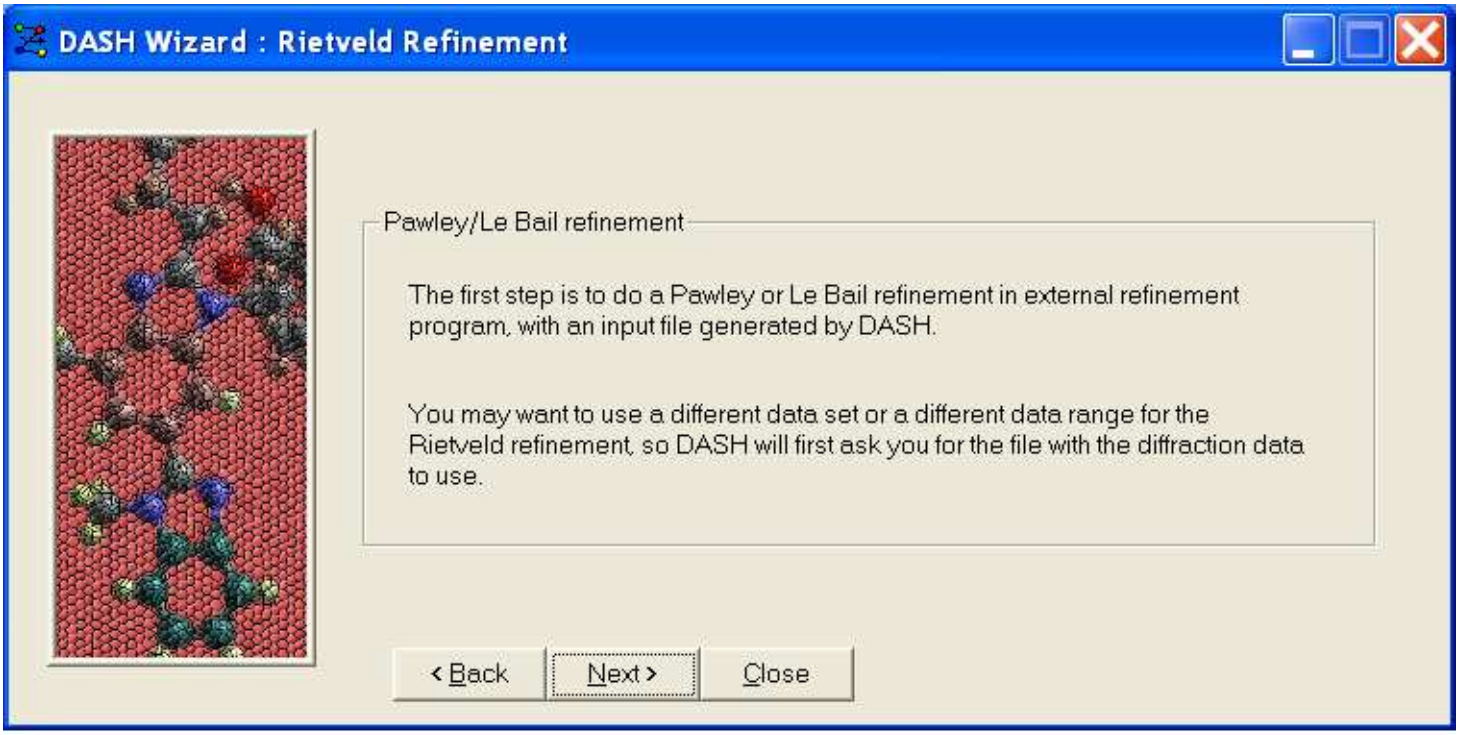
12.2.2 Rigid-Body Refinement Steps

- When the *Rigid-Body Rietveld Refinement* dialogue box is launched, the **Global isotropic temperature factor** check box is automatically selected, and hitting **Refine** will carry out the first step of the Rietveld refinement. This step will not change the crystal structure, only the thermal parameters are affected.
- The two most likely remaining candidates for refinement are the valence angles and the torsion angles. The temperature factors tend to correlate with all other parameters, and it therefore best *not* to refine the temperature factors in combination with other parameters. Therefore, the next step is to deselect the **Global isotropic temperature factor** check box and to click on the **Set** button for the valence angles, which will select all angles (except those involving Hydrogens) to be refined.
- Next, deselect all angles by pressing the **Clear** button, then select all torsion angles (**Set**) and refine those.
- Depending on how much the Chi-sqd values have changed, it may now be necessary to refine the positions and orientations of the Z-matrices again (keeping all bond lengths, angles and torsion angles fixed).
- Depending on the quality of your data (it must be good) and the quality of your initial model (if you have reason to suspect it contains errors), you may try to refine some or all of the bond lengths.
- Depending on how much the Chi-sqd values changed, you can return to the isotropic temperature factor again, and repeat the whole cycle.

12.3 Preparation of data for Rietveld Refinement using TOPAS, GSAS or RIETAN

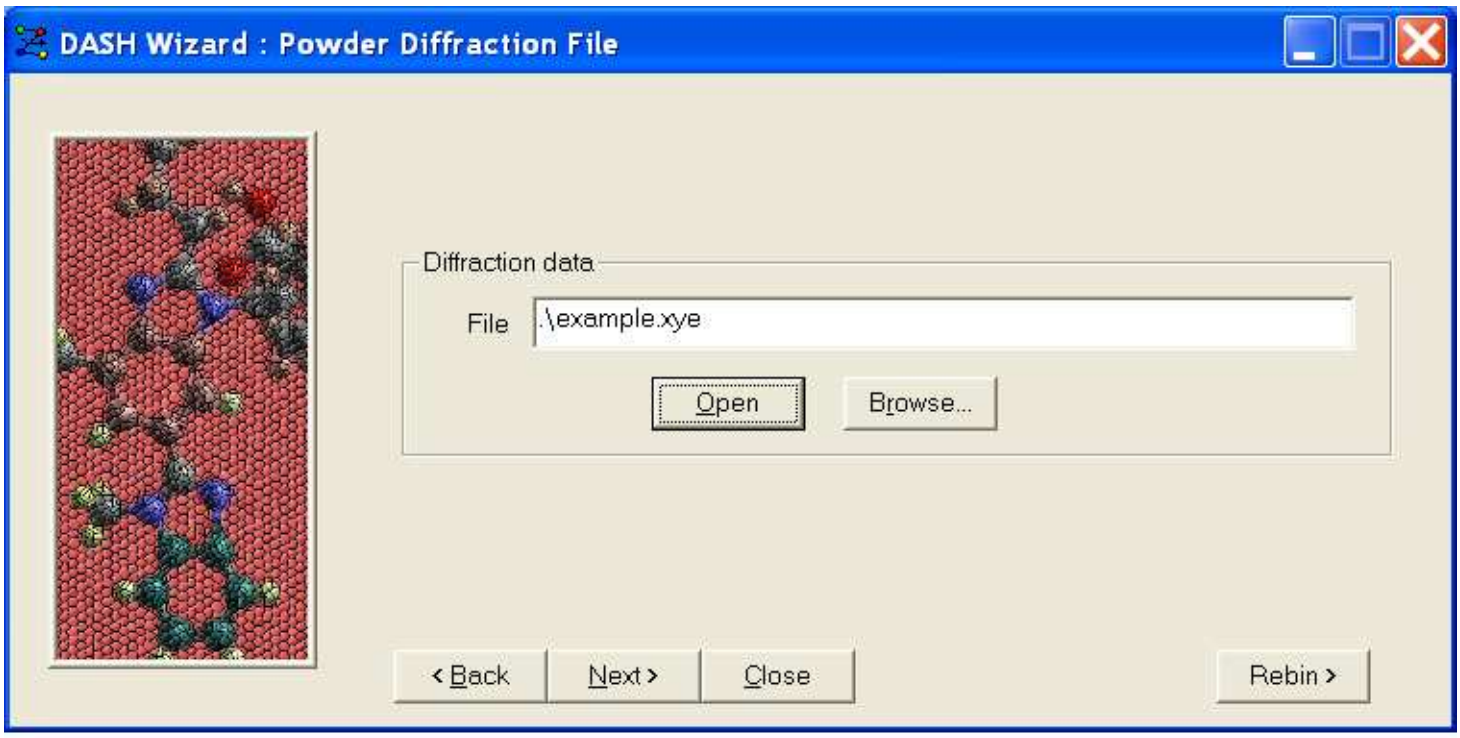


After choosing the refinement package you wish to use within the Rietveld Refinement Wizard and selecting the required `.sdi` and crystal structure files, click **Next >**.

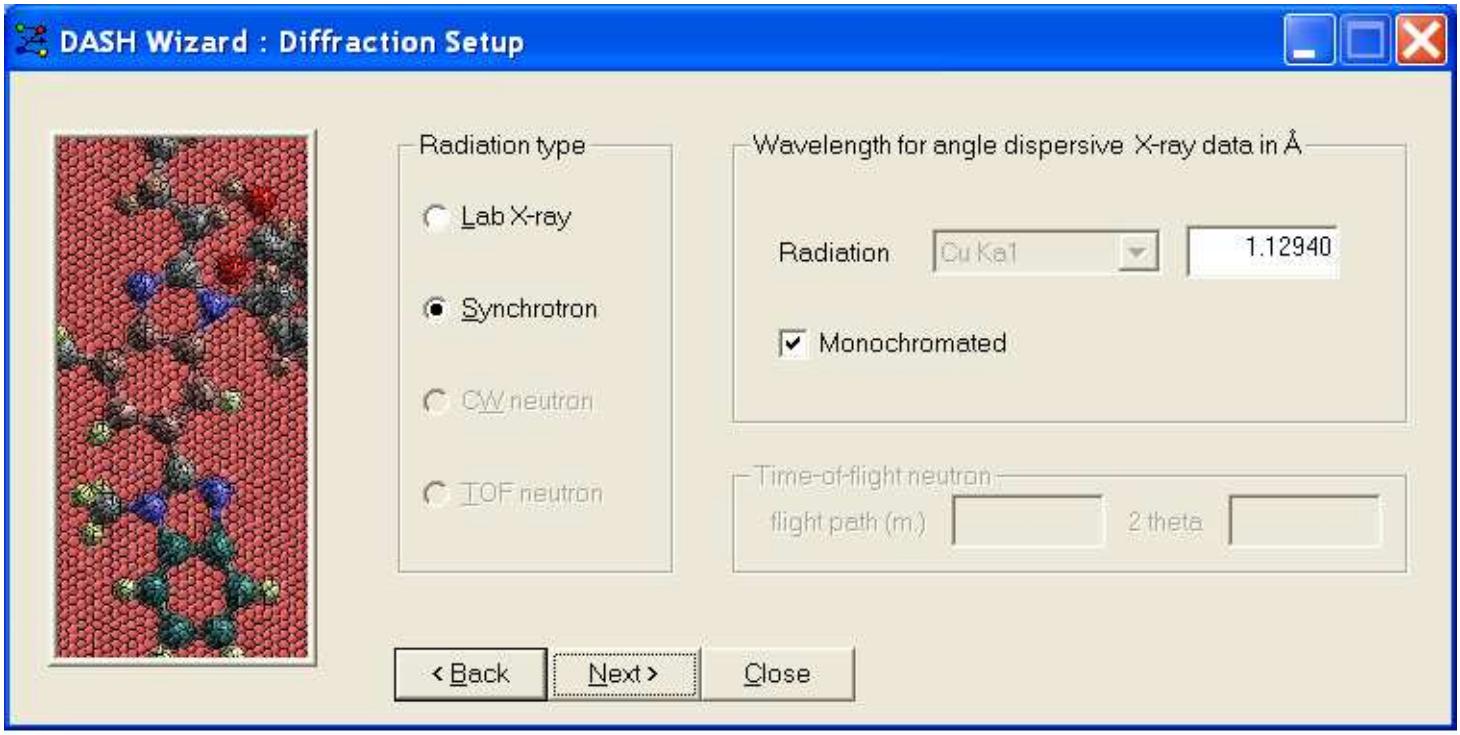


In order to perform the Rietveld refinement, it is necessary to do a Pawley or Le Bail fit within your chosen refinement package using an input file generated by DASH. It may be advantageous to use a

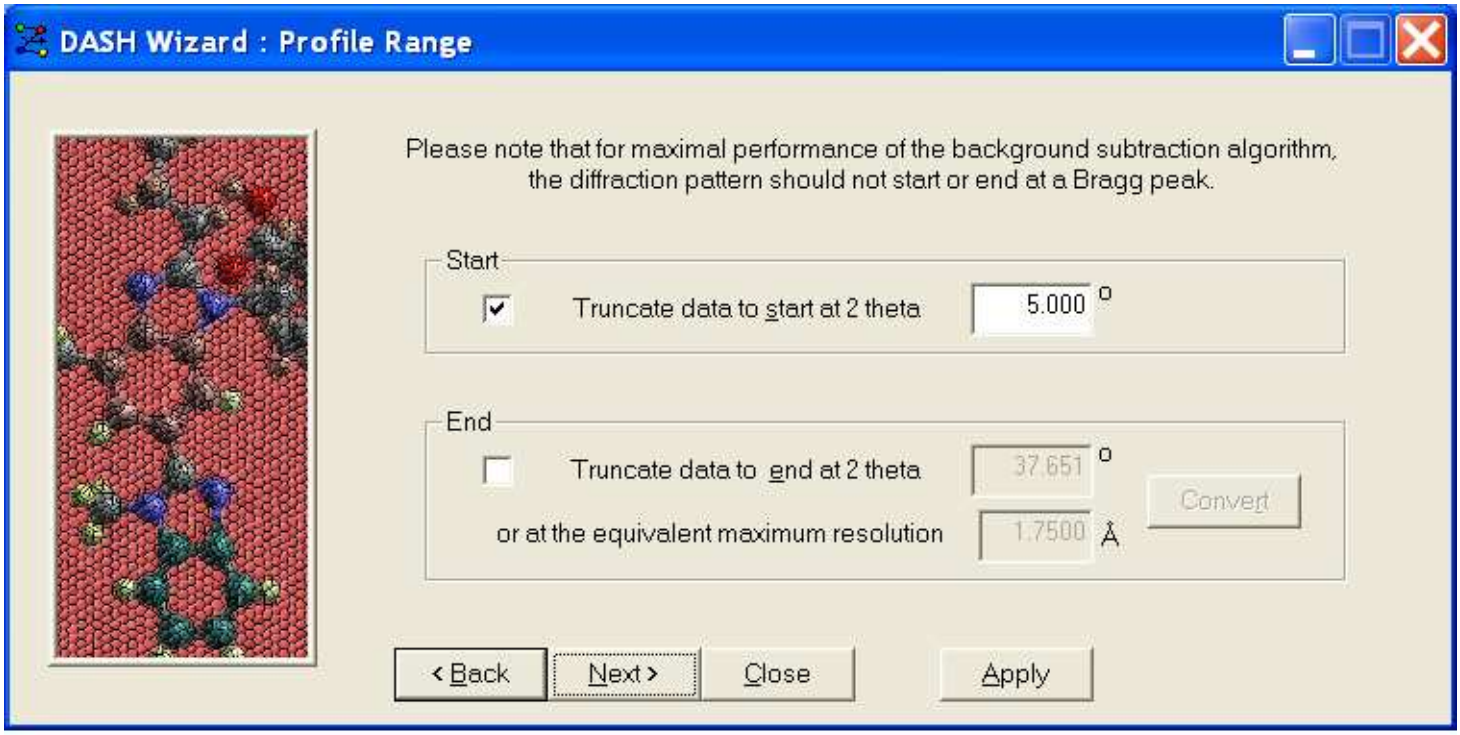
different data set or data range for the Rietveld refinement to that used for structure solution, so it will now be necessary to read in the diffraction data, click **Next >**.



Click **Browse** to find the diffraction data file that you want to use for Rietveld refinement and then click on **Next >** to read the data into DASH.



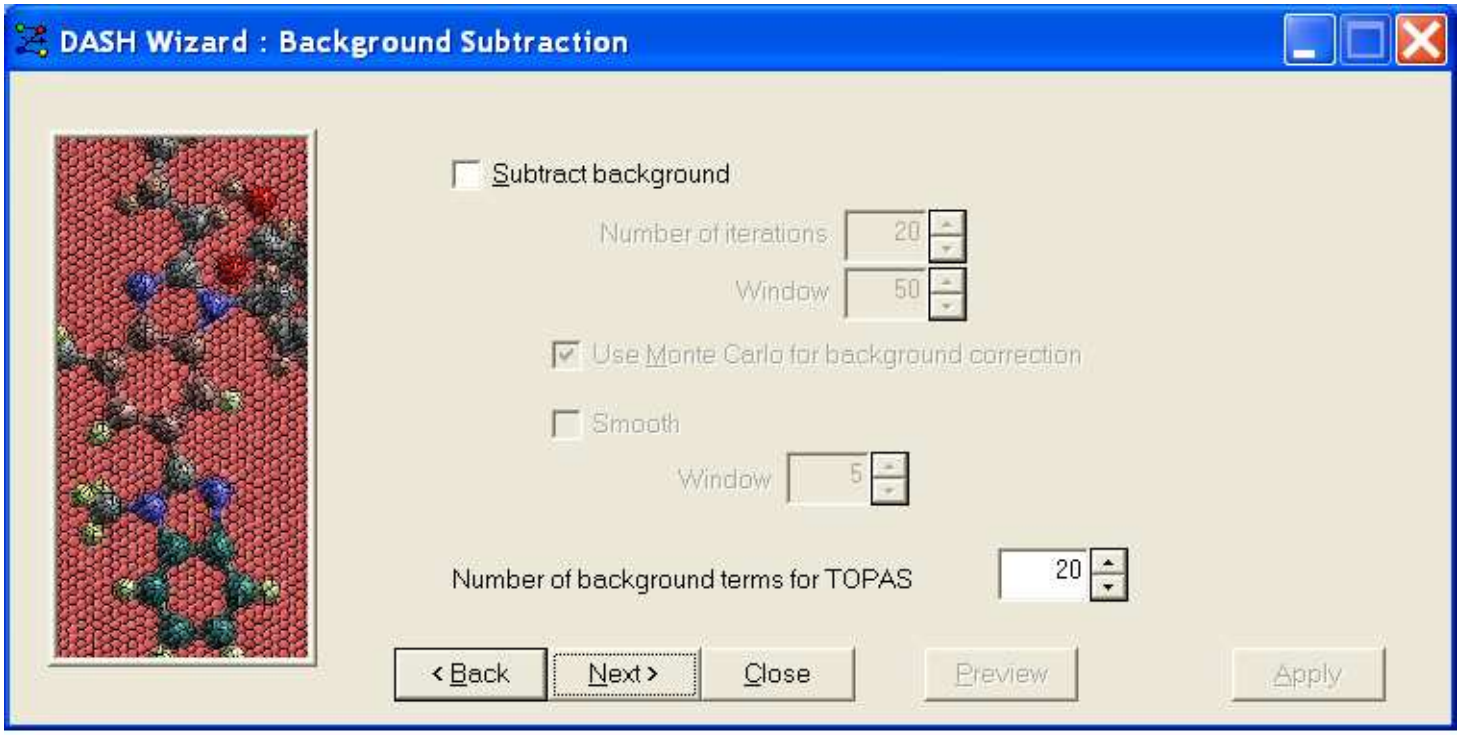
- Check that the radiation type and wavelength specified are correct.
- If the data are monochromated, ensure that the **Monochromated** check box is ticked.
- If you are going to use GSAS for the refinement you have the opportunity to load in a GSAS `.ins` file. You may want to do this if you have measured instrument parameters that you would like to use in the refinement. Check the **Load .ins file** check box and browse to the location of the file.
- Click **Next >** to continue.



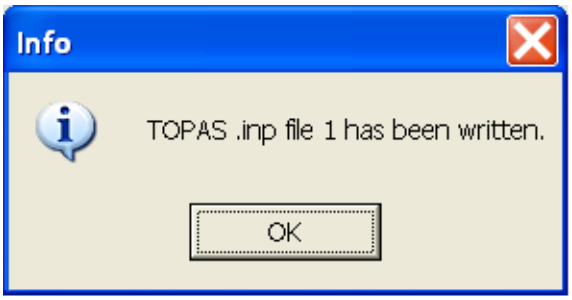
The data range to be used can be modified at this point, but it is usual to utilise as much of the diffraction pattern as possible during Rietveld refinement, hence the data truncation option has been switched off by default. If you wish to truncate the data, enter the range to be used.
Click **Next >** to proceed.

12.4 Refinement using TOPAS

12.4.1 Preparation of Data for TOPAS Rietveld Refinement

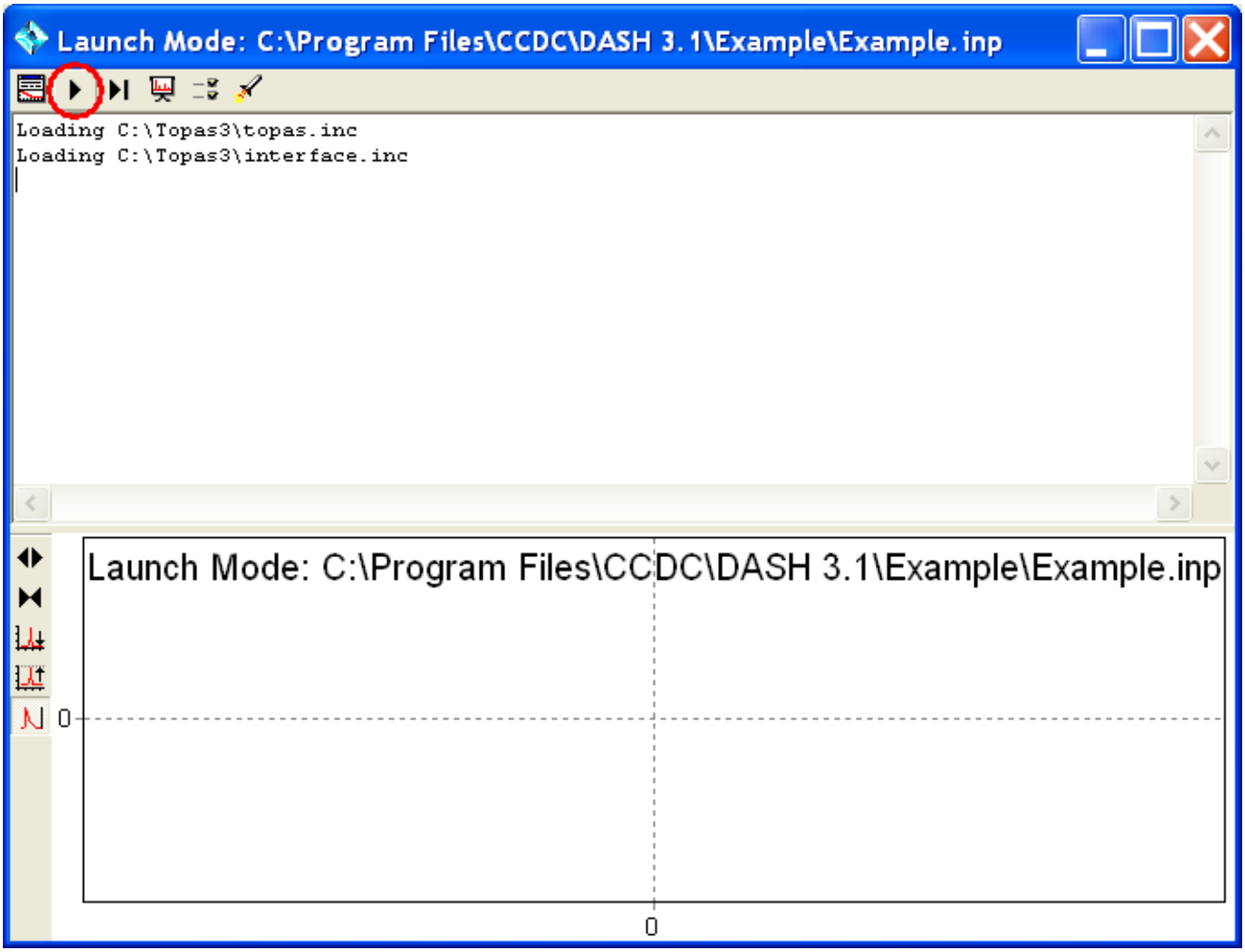


Complete the steps of data preparation given (see Section 12.3, page 141). The final step before sending the pattern to TOPAS is the Background Subtraction. This can be done within DASH, but it is usually better to allow TOPAS to perform the background subtraction (using 20 background terms as default).
Click **Next >.** A dialog will inform you when the first `.inp` file has been written and hence is ready to be loaded into TOPAS.



Once TOPAS is open, launch the Kernal Mode (**Launch-> Kernal Mode**) and set the `.inp` file to the `.inp` file written by DASH (**Launch->Set INP file**). To run the refinement hit the **<play>** button

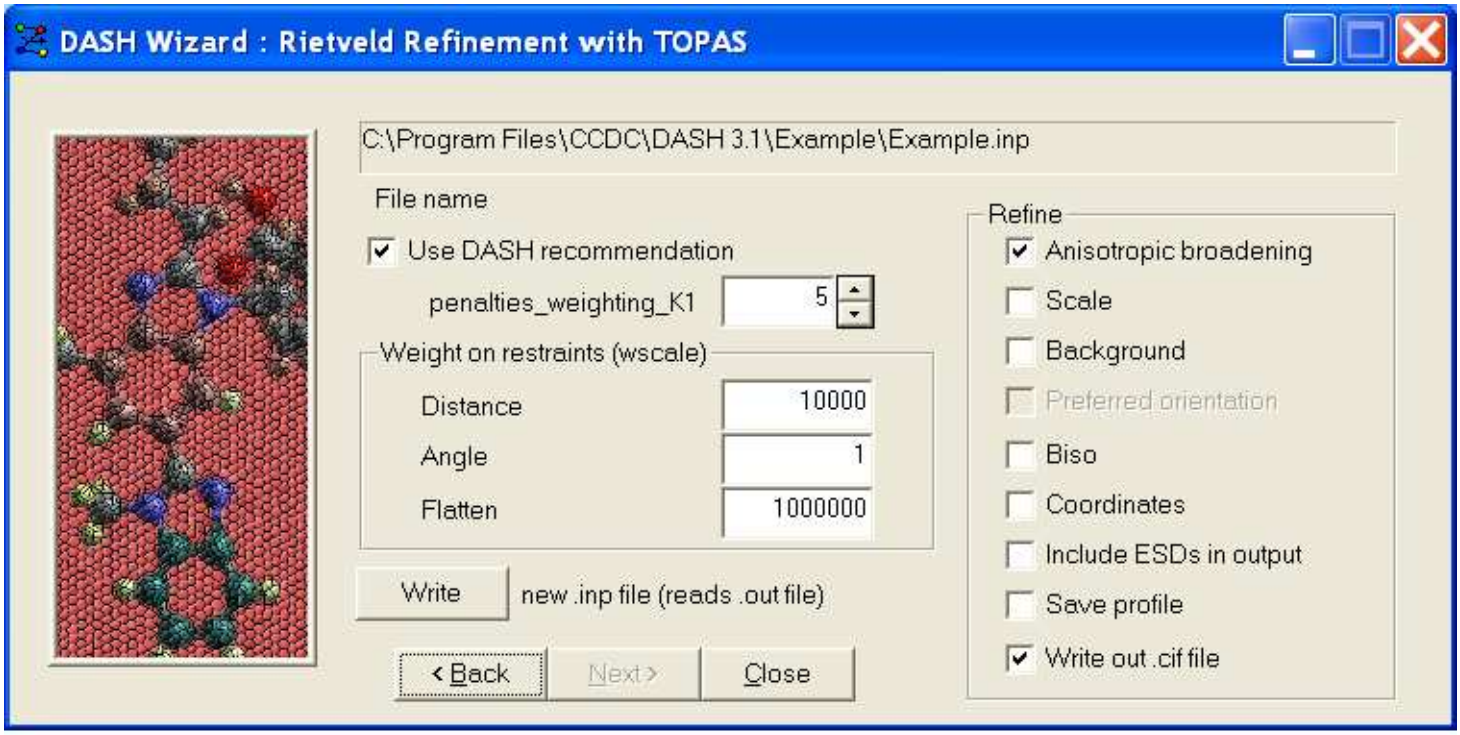
(circled in red in the following screenshot) in the window called *Launch Mode*.



Once the cycle is complete, TOPAS will ask you whether you wish to update the `.inp` file with an .out file. Hit **No** and return to DASH.

12.4.2 TOPAS Rietveld Refinement

The DASH interface for TOPAS will now guide you through the process of Rietveld refinement. At any point you may exit the refinement process by clicking on **Close**.



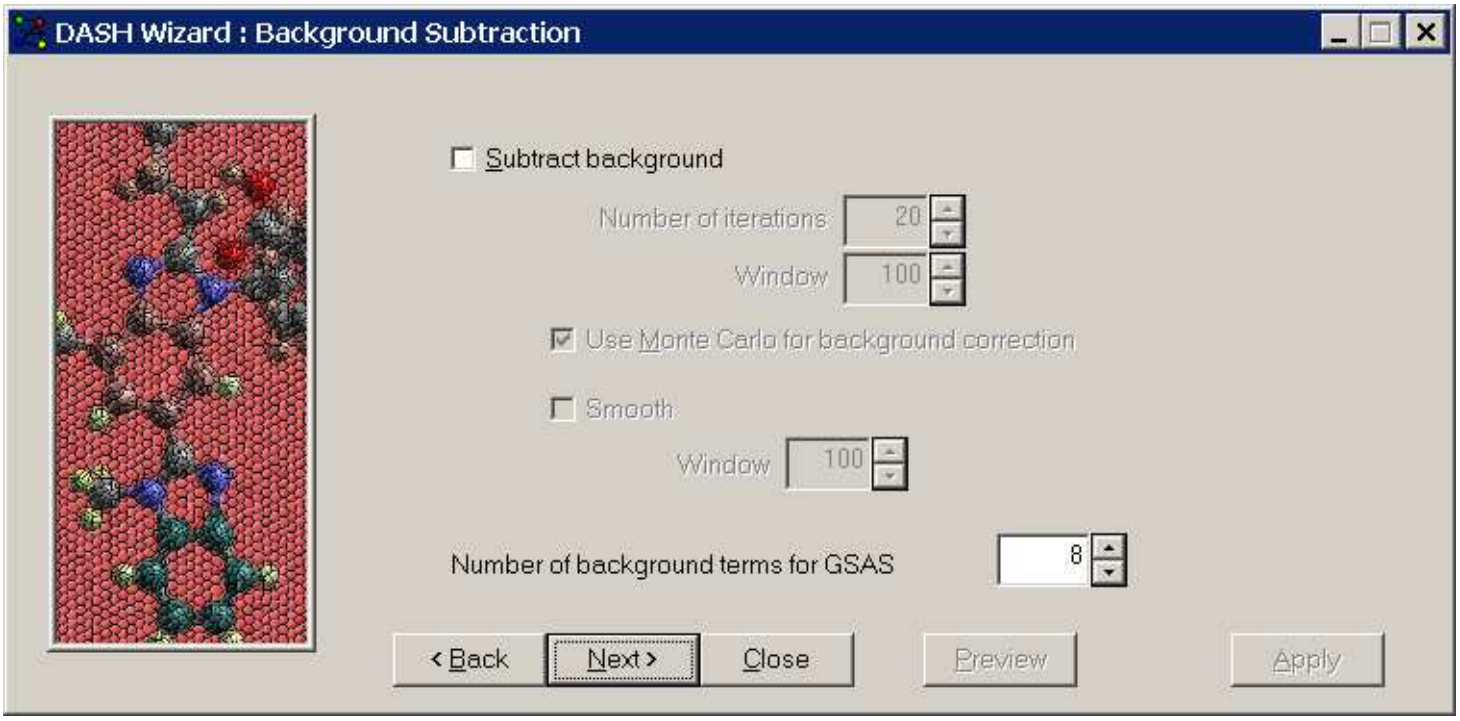
The *Rietveld Refinement with TOPAS* wizard window will be displayed with the **Anisotropic broadening** check box ticked. This is the first parameter to be refined.

- Hit **Write** and a new .inp file will be written, ready to be loaded into TOPAS.
- Return to TOPAS and hit the **<play>** button of the *Launch Mode* window. You will be asked whether you wish to update the .inp file with the .out file. Hit **No** and return to DASH.
- Repeat the above cycle until all the parameters have been refined (six .inp files should be written for a standard refinement). In the final cycle a `.cif` file of the solution will be written by TOPAS.

Note: Default values for **Weights on Restraints** are provided but can be customised.

12.5 Refinement Using GSAS

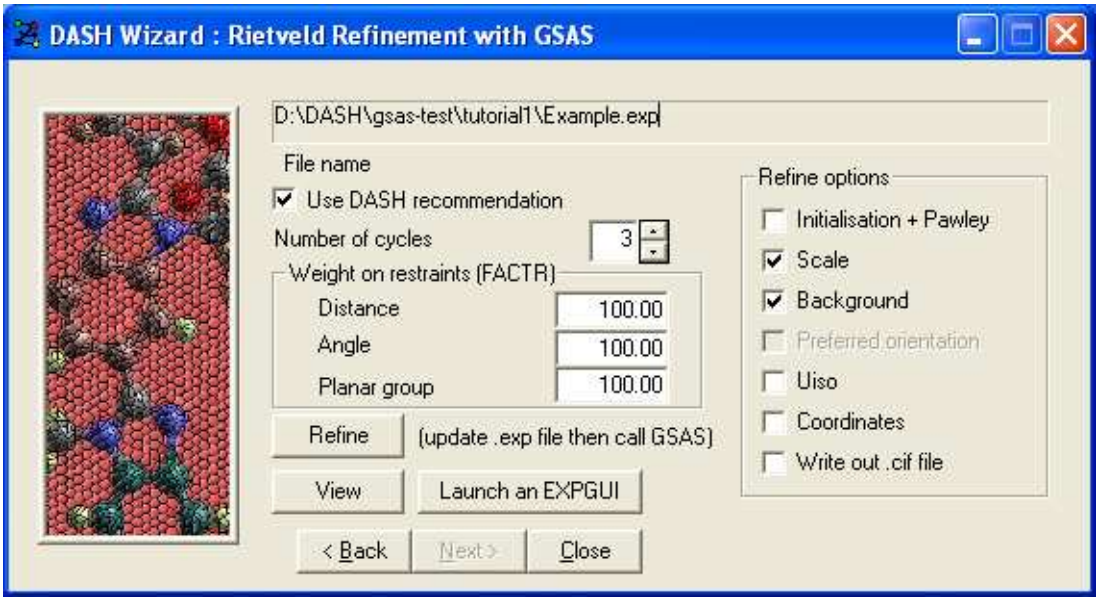
12.5.1 Preparation of Data for GSAS Rietveld Refinement



Complete the steps of data preparation given (see Section 12.3, page 141). The final step before sending the pattern to GSAS is the Background Subtraction. This can be done within DASH, but it is usually better to allow GSAS to perform the background subtraction (using eight background terms as default).

- Click **Next >** to save the GSAS `.exp` file. The Pawley fit will now automatically be performed within GSAS, follow the on-screen instructions when prompted. Once the fit has been done, a plot of the diffraction profile, the fit and the difference profile will be shown.
- Close this plot by clicking on **File-> Quit**. Return to DASH where you can continue with the GSAS Rietveld Refinement.

12.5.2 GSAS Rietveld Refinement



The DASH interface for GSAS will now guide you through the process of Rietveld refinement. At any point you may:

- view the structure (e.g. in *Mercury*) by clicking on **View**,
- switch to using *EXPGUI* for refinement by clicking on **Launch an EXPGUI**
- exit the refinement process by clicking on **Close**.

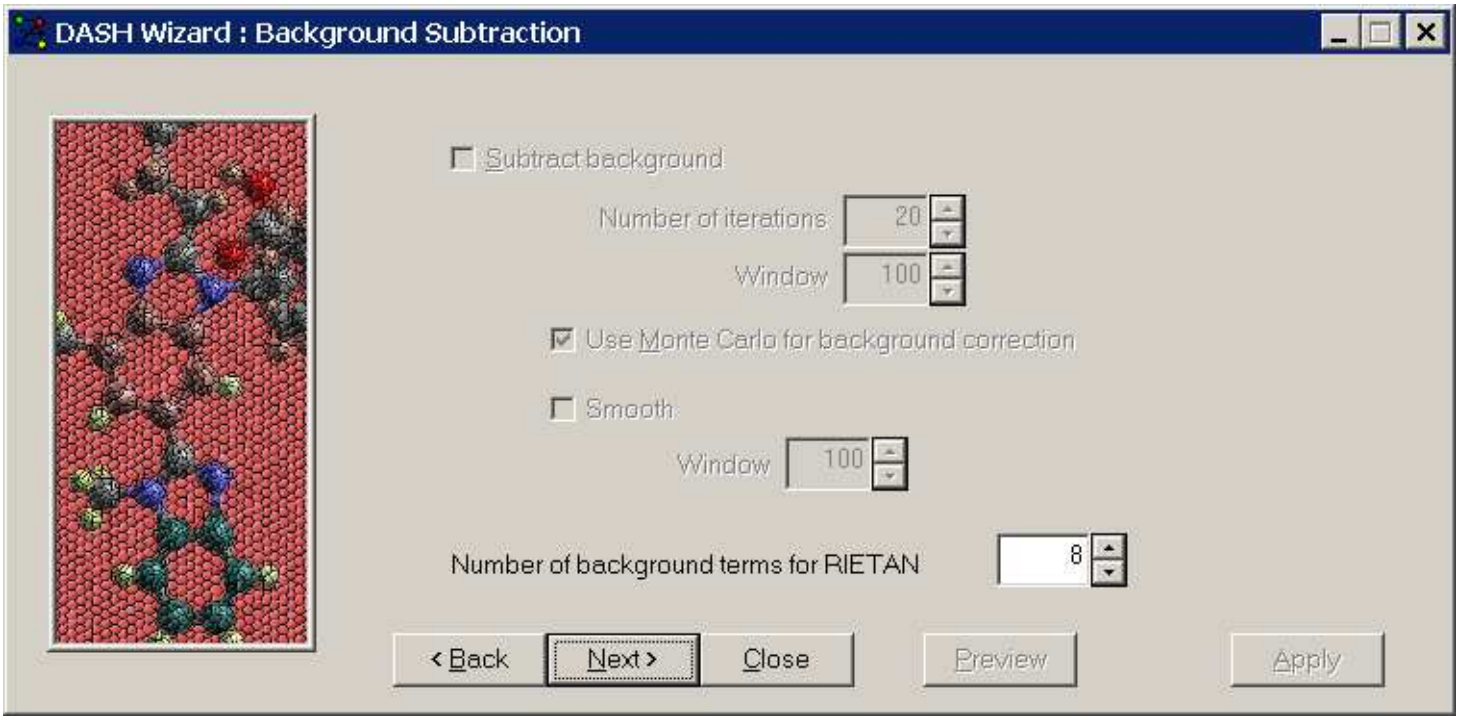
The right-hand side of the dialogue box shows the current refinement options - to start with only two boxes are ticked to show that only the **Scale** and **Background** terms will be refined.

- Click on the **Refine** button and the `.exp` file will be automatically updated then submitted to GSAS for the first step of refinement. When the cycle of refinement has completed you will be returned to the DASH interface.
- The *Refine options* have been updated such that a **Uiso** parameter will be refined in the next cycle of refinement. Continue this process of refinement until all the checkboxes have been ticked.
- The final cycle of refinement will be followed by output of the final structure in `.cif` format.

Note: Default values for **Weights on Restraints (FACTR)** are provided but can be altered.

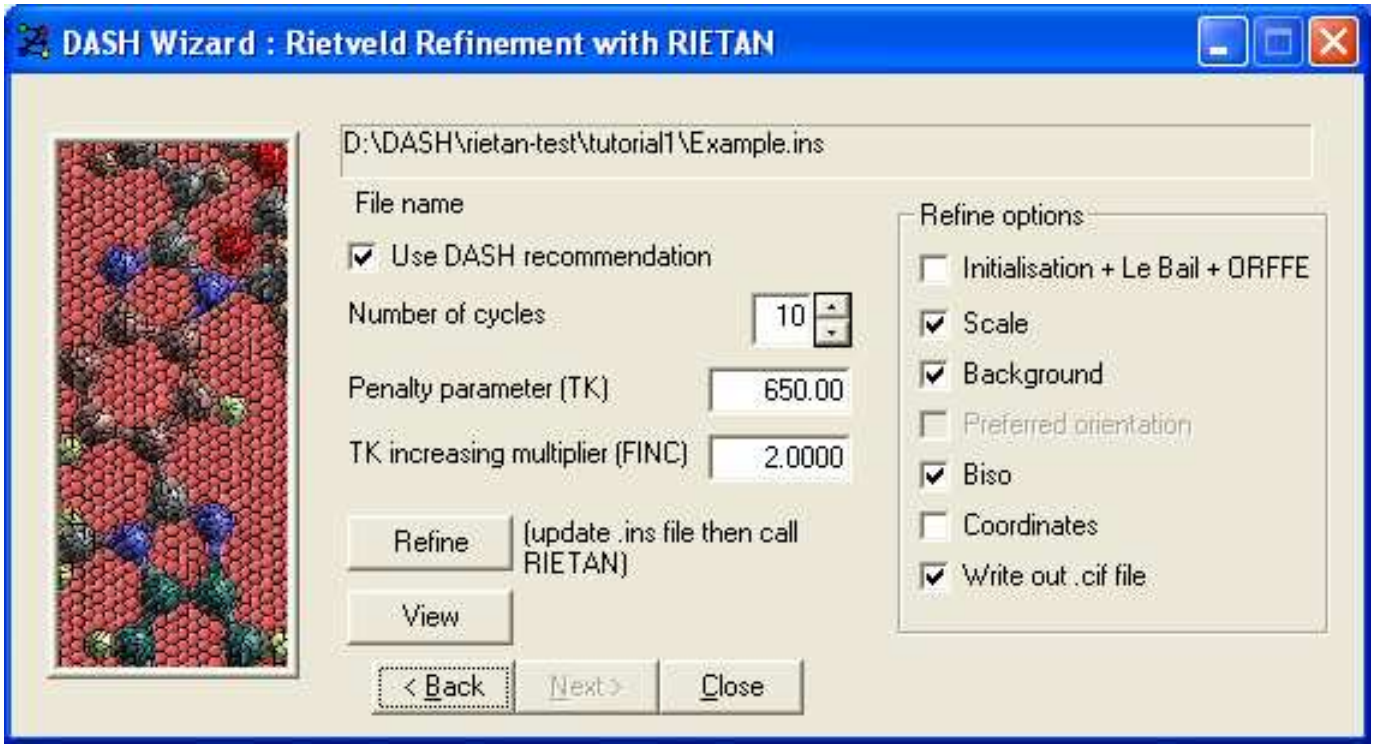
12.6 Refinement Using RIETAN

12.6.1 Preparation of Data for RIETAN Rietveld Refinement



Complete the steps of data preparation given (see Section 12.3, page 141). The final step, before sending the pattern to RIETAN, is to choose the number of terms to be used by RIETAN for the background subtraction; the default value is eight.
Click **Next >** to save RIETAN's `.ins` file. The pattern fitting will now be performed automatically within RIETAN. Once the initial fit has been done, a plot of the diffraction profile, the fit and the difference profile will be shown in gnuplot graph. The fitting of the pattern is a two step process: closing the gnuplot window (**File->Exit**) will start the second step of the fitting. Once complete, RIETAN will prompt you to save the `.ins` file.

12.6.2 RIETAN Rietveld Refinement



The DASH interface for RIETAN will now guide you through the process of Rietveld refinement. At any point you may:

- view the structure (e.g. in *Mercury*) by clicking on **View**
- exit the refinement process by clicking on **Close**.

The right-hand side of the dialogue box shows the current refinement options - to start with, three boxes are ticked, showing that only the **Scale**, **Background** and **Biso** terms will be refined.

- Click on the **Refine** button and the `.ins` file will be automatically updated, then submitted to RIETAN for the first step of refinement. When the cycle of refinement has completed you will be prompted to save the `.ins` file before being returned to the DASH interface.
- The *Refine options* will have been updated to include the atomic coordinates for the final cycle of refinement. Click **Refine** to complete the refinement.

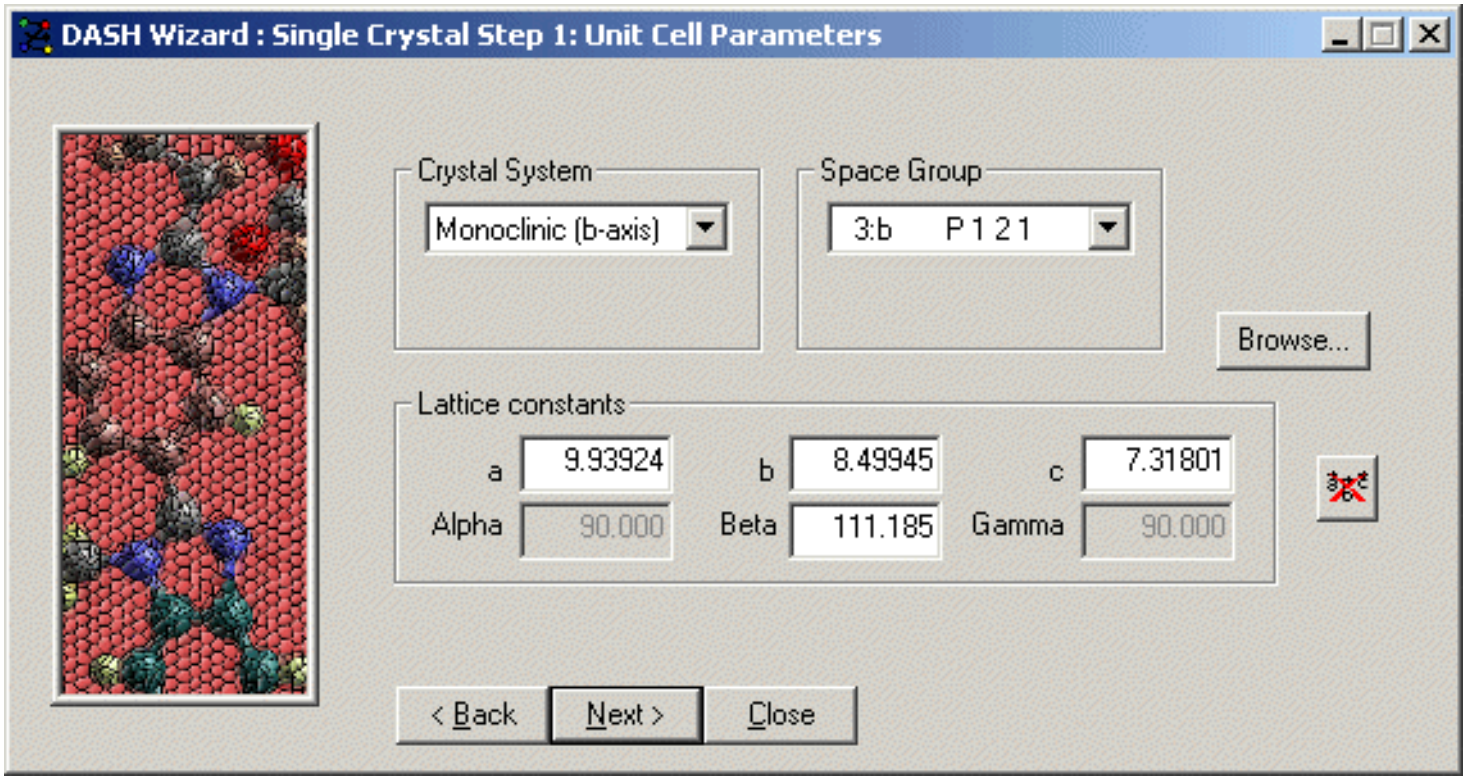
Note: Default values for the **Number of cycles, Penalty parameter (TK)** and **TK increasing multiplier (FINC)** are provided but can be altered.

Note Output from RIETAN is written to a `.lst` file should you wish to look up the reported *Rwp* and *Rp* values for the refinement cycles.

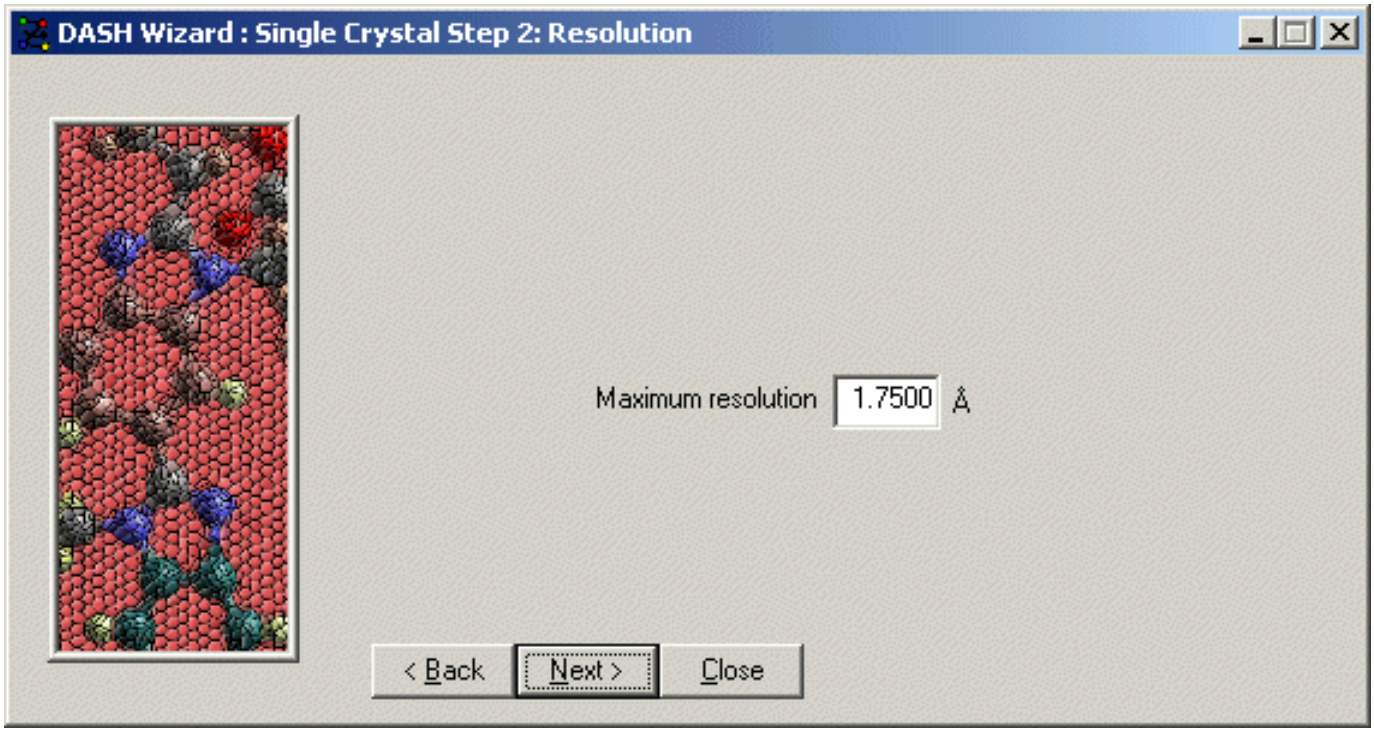
13 PREPARATION OF SINGLE CRYSTAL DATA

If you have poor single crystal data then it may be possible to solve the structure within DASH using the simulated annealing protocol. This is often successful in cases such as limited hkl ranges from high pressure cells, or just poorly diffracting crystals which do not give enough data for direct methods phasing. DASH requires a file containing the h, k, l, F^2 and $\mathtt{s}(F^2)$ values in the SHELX format and a molecular model. Once the hkl data have been read in, the structure solution proceeds as for powder diffraction.

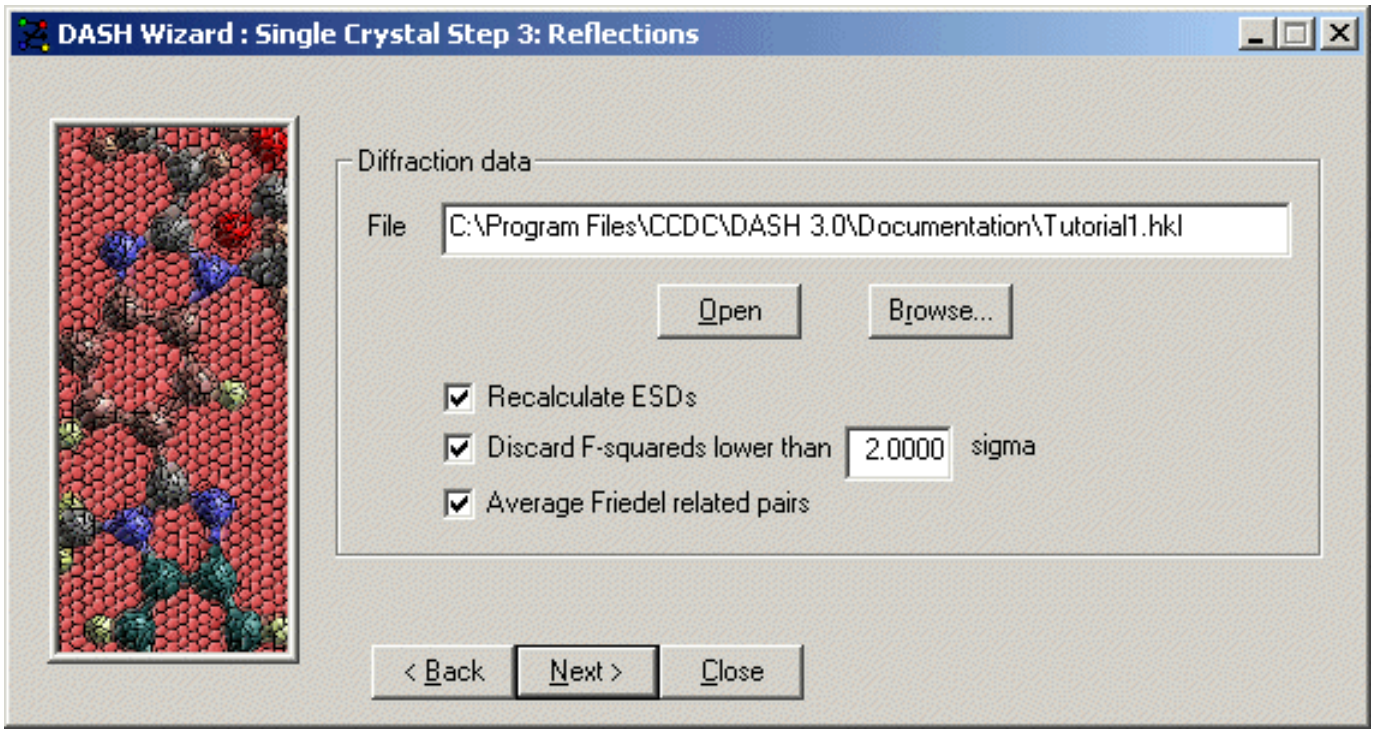
- Select **Preparation of single crystal data** from the first DASH Wizard (see Section 2.10, page 21) and click **Next >**.
- Enter the crystal system, space group and unit cell parameters in the boxes provided:



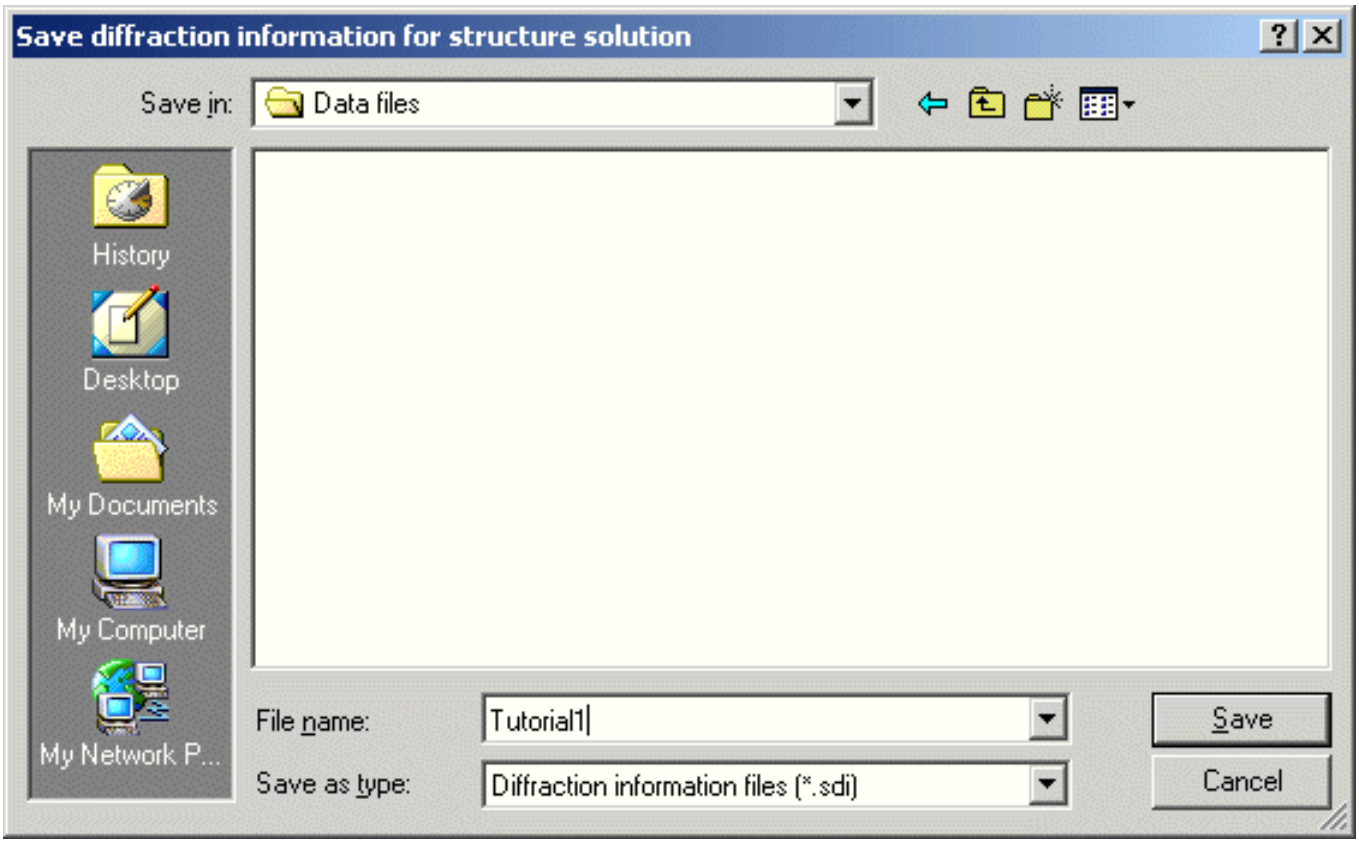
- Click **Next >**.
- This window allows the maximum resolution of the data to be set. In most cases the default resolution of 1.75 Å will be appropriate:



- Click **Next >**.
- Open the file containing the h, k, l, F^2 and $\mathbf{s}(F^2)$ values. The *.hkl* file is expected to be in SHELX format:



- There are three options available for processing the data, and in most cases it is appropriate to use the default values:
  - Recalculate ESDs
  - Discard F^2 lower than 2.0 sigma
  - Average Friedel related pairs
- Click **Next >**.
- Enter the file name of an `.sdi` file where the information required for structure solution will be saved:



- This will bring up the *DASH Wizard : Molecular Z-Matrices* window. Proceed with entering a molecular model and setting up simulated annealing runs as for normal powder data (see Section 10, page 101).

14 APPENDICES

14.1 Appendix A: Programs for Indexing and Cell Reduction

A good starting point for acquiring programs and advice is the Collaborative Computational Project Number 14 (CCP14): *http://www.ccp14.ac.uk*.

In particular, *http://www.ccp14.ac.uk/solution/indexing/* lists available software for powder indexing. One simple way to cover all of the tried and tested indexing approaches is to retrieve and install the CRYSFIRE indexing suite, which packages together a number of indexing programs under a common interface. The CHEKCELL program may prove a useful tool for discriminating between multiple solutions returned by an indexing program.

In our experience, DICVOL has proven to be a highly effective program for indexing powders. Note, however, that it is not especially tolerant of spurious input lines. For example, the insertion of a single spurious line into an otherwise accurate set of input lines can cause the solution to be missed. In contrast, the absence of one or two correct lines from the input file does not necessarily mean that the indexing solution will not be found.

14.2 Appendix B: Programs for Building 3D Molecules

Popular choices are:

- Chem3D Ultra - *http://www.cambridgesoft.com/products/*
- WebLabViewer - *http://www.accelrys.com/viewer/*
- Cerius^{2} - *http://www.accelrys.com/cerius2/index.html*
- SYBYL- *http://www.tripos.com/software/sybyl.html*
- SPARTAN - *http://www.wavefun.com/software/software.html*
- CORINA - *http://www2.ccc.uni-erlangen.de/software/corina/*
- CONCORD - *http://www.tripos.com/software/concord.html*

It must be emphasised that you should examine the model created by such programs carefully. It is quite possible to build a model that has poor bond-lengths and angles, which actually prevent you from solving the structure. If in doubt always check the *Cambridge Structural Database*.

14.3 Appendix C: Definitions of DASH Figures of Merit

Figures of merit in DASH

Figures of merit for the Pawley fit to the profile:

$$R_{wp} = \left[\frac{\sum_i w_i \left(y_{i(obs)} - y_{i(calc)} \right)^2}{\sum_i w_i \left(y_{i(obs)} - y_{i(back)} \right)} \right]^{\frac{1}{2}}$$

Weighted profile R-factor

$$R_E = \left[\frac{N - P + C}{\sum_i w_i \left(y_{i(obs)} - y_{i(back)} \right)} \right]^{\frac{1}{2}}$$

Expected profile R-factor

Where (N -P +C) = (number of observations) - (number of parameters) + (number of constraints).

$$c^2 = \frac{\sum_i w_i \left(y_{i(obs)} - y_{i(calc)} \right)^2}{(N - P + C)}$$

Profile c^2

Figures of merit for the Simulated Annealing Run

$$c^2 = \sum_h \sum_k \left[\left(I_h - c|F_h|^2 \right) \left(V^{-1} \right)_{hk} \left(I_k - c|F_k|^2 \right) \right]$$

c^2 for the integrated intesities

where $I_{h,k}$ is the extracted intensity from a Pawley refinement of the diffraction pattern, V_{hk} is the covariance matrix from the Pawley refinement, c is the scale factor and $F_{h,k}$ is the calculated structure factor from the current trial structure.

$$c^2 = \frac{\sum_i w_i \left(y_{i(obs)} - y_{i(calc)} \right)^2}{\left(N - P + C \right)}$$

Profile c^2

14.4 Appendix D: Frequency of Occurrence of Space Groups

This appendix lists space groups by their frequency of occurrence in organic and organometallic crystal structures.

14.4.1 D.1 Space Groups Listed by Frequency of Occurrence

This table lists all space groups that occur at least 40 times in the Cambridge Structural Database (CSD), arranged in descending order of frequency. Since there were already over 250,000 structures in the CSD when this list was compiled, any space group not included in the table is very uncommon. Only the symbol for the standard setting is given for each space group in the list.

Number	Symbol	Occurrences	Number	Symbol	Occurrences
14	$P2_1/c$	73151	2	*P-1*	41639
19	$P2_12_12_1$	18216	15	*C2/c*	15150
4	$P2_1$	11572	61	*Pbca*	7768
33	$Pna2_1$	3157	62	*Pnma*	3128
9	*Cc*	2075	1	*P1*	1848
60	*Pbcn*	1841	5	*C2*	1702
29	$Pca2_1$	1474	11	$P2_1/m$	1350
13	*P2/c*	1064	12	*C2/m*	1053
148	*R-3*	1044	18	$P2_12_12$	970
7	*Pc*	760	56	*Pccn*	720
43	*Fdd2*	684	88	$I4_1/a$	671
92	$P4_12_12$	522	20	$C222_1$	393
36	$Cmc2_1$	362	64	*Cmca*	334
82	*I-4*	330	176	$P6_3/m$	325
146	*R3*	311	96	$P4_32_12$	290

114	*P-42$_1$c*	289	57	*Pbcm*	285
86	*P4$_2$/n*	275	161	*R3c*	271
63	*Cmcm*	270	76	*P4$_1$*	240
167	*R-3c*	236	205	*Pa3*	226
152	*P3$_1$2$_1$*	222	85	*P4/n*	213
41	*Aba2*	211	147	*P-3*	194
31	*Pmn2$_1$*	188	70	*Fddd*	180
58	*Pnnm*	173	144	*P3$_1$*	168
52	*Pnna*	166	173	*P6$_3$*	149
45	*Iba2*	143	198	*P2$_1$3*	143
169	*P6$_1$*	132	145	*P3$_2$*	131
8	*Cm*	126	87	*I4/m*	122
122	*I-4$_2$d*	114	78	*P4$_3$*	113
154	*P3$_2$2$_1$*	106	165	*P-3c1*	100
72	*Ibam*	99	59	*Pmmn*	92
68	*Ccca*	92	170	*P6$_5$*	90
155	*R3$_2$*	87	142	*I4$_1$/acd*	86
110	*I4$_1$cd*	85	130	*P4/ncc*	85
166	*R-3m*	84	54	*Pcca*	81
129	*P4/nmm*	79	160	*R3m*	78
55	*Pbam*	74	34	*Pnn2*	72
113	*P-421m*	71	143	*P3*	70
163	*P-3$_1$c*	62	225	*Fm3m*	62
121	*I-4$_2$m*	57	80	*I4$_1$*	56
79	*I4*	55	81	*P-4*	55
217	*I-4$_3$m*	55	23	*I222*	54
3	*P2*	53	10	*P2/m*	53
186	*P6$_3$mc*	53	118	*P-4n2*	49
126	*P4/nnc*	49	127	*P4/mbm*	49
194	*P6$_3$/mmc*	49	26	*Pmc2$_1$*	48

159	$P3_1c$	48	73	*Ibca*	47
178	$P6_122$	47	94	$P4_22_12$	46
136	$P4_2/mnm$	46	32	*Pba2*	44
17	$P222_1$	43	40	*Ama2*	42

14.4.2 D.2 Chiral (Sohnke) Space Groups Listed by Frequency of Occurrence

This table lists all Sohnke space groups (i.e. space groups in which enantiomerically pure substances can crystallise) that occur at least 40 times in the Cambridge Structural Database (CSD), arranged in descending order of frequency. Since there were over 250,000 structures in the CSD when this lost was compiled, any space group not in the table is very uncommon. Only the symbol for the standard setting is given for each space group in the list.

Number	Symbol	Occurrences	Number	Symbol	Occurrences
19	$P2_12_12_1$	18216	4	$P2_1$	11572
1	*P1*	1848	5	*C2*	1702
18	$P2_12_12$	970	92	$P4_12_12$	522
20	$C222_1$	393	146	*R3*	311
96	$P4_32_12$	290	76	$P4_1$	240
152	$P3_12_1$	222	144	$P3_1$	168
173	$P6_3$	149	198	$P2_13$	143
169	$P6_1$	132	145	$P3_2$	131
78	$P4_3$	113	154	$P3_22_1$	106
170	$P6_5$	90	155	$R3_2$	87
143	*P3*	70	80	$I4_1$	56
79	*I4*	55	23	*I222*	54
3	*P2*	53	178	$P6_122$	47
94	$P4_22_12$	46	17	$P222_1$	43

14.4.3 D.3. Space Group Listing for DASH.

The *Entry Number* is an internal identifier used by DASH. The corresponding space group number, setting and symbol are shown in the second column of the table. Within a DASH `.sdi` file, the space group is stored as:

```
SpaceGroup entry_number sg#:setting symbol
```
For example:

```
SpaceGroup   39      4:b       P 1 21 1
or
SpaceGroup   52      9:b3      I 1 a 1
```

Entry number	SG#:setting	Symbol
1	1	P 1
2	2	P -1
3	3:a	P 2 1 1
4	4:a	P 21 1 1
5	5:a1	B 2 1 1
6	5:a2	C 2 1 1
7	5:a3	I 2 1 1
8	6:a	P m 1 1
9	7:a1	P b 1 1
10	7:a2	P n 1 1
11	7:a3	P c 1 1
12	8:a1	B m 1 1
13	8:a2	C m 1 1
14	8:a3	I m 1 1
15	9:a1	B b 1 1
16	9:a2	C n 1 1
17	9:a3	I c 1 1
18	9:-a1	C c 1 1
19	9:-a2	B n 1 1
20	9:-a3	I b 1 1
21	10:a	P 2/m 1 1
22	11:a	P 21/m 1 1
23	12:a1	B 2/m 1 1
24	12:a2	C 2/m 1 1
25	12:a3	I 2/m 1 1
26	13:a1	P 2/b 1 1
27	13:a2	P 2/n 1 1
28	13:a3	P 2/c 1 1
29	14:a1	P 21/b 1 1
30	14:a2	P 21/n 1 1
31	14:a3	P 21/c 1 1
32	15:a1	B 2/b 1 1

33	15:a2	C 2/n 1 1
34	15:a3	I 2/c 1 1
35	15:-a1	C 2/c 1 1
36	15:-a2	B 2/n 1 1
37	15:-a3	I 2/b 1 1
38	3:b	P 1 2 1
39	4:b	P 1 21 1
40	5:b1	C 1 2 1
41	5:b2	A 1 2 1
42	5:b3	I 1 2 1
43	6:b	P 1 m 1
44	7:b1	P 1 c 1
45	7:b2	P 1 n 1
46	7:b3	P 1 a 1
47	8:b1	C 1 m 1
48	8:b2	A 1 m 1
49	8:b3	I 1 m 1
50	9:b1	C 1 c 1
51	9:b2	A 1 n 1
52	9:b3	I 1 a 1
53	9:-b1	A 1 a 1
54	9:-b2	C 1 n 1
55	9:-b3	I 1 c 1
56	10:b	P 1 2/m 1
57	11:b	P 1 21/m 1
58	12:b1	C 1 2/m 1
59	12:b2	A 1 2/m 1
60	12:b3	I 1 2/m 1
61	13:b1	P 1 2/c 1
62	13:b2	P 1 2/n 1
63	13:b3	P 1 2/a 1
64	14:b1	P 1 21/c 1
65	14:b2	P 1 21/n 1
66	14:b3	P 1 21/a 1
67	15:b1	C 1 2/c 1
68	15:b2	A 1 2/n 1
69	15:b3	I 1 2/a 1
70	15:-b1	A 1 2/a 1
71	15:-b2	C 1 2/n 1
72	15:-b3	I 1 2/c 1
73	3:c	P 1 1 2

74	4:c	P 1 1 21
75	5:c1	A 1 1 2
76	5:c2	B 1 1 2
77	5:c3	I 1 1 2
78	6:c	P 1 1 m
79	7:c1	P 1 1 a
80	7:c2	P 1 1 n
81	7:c3	P 1 1 b
82	8:c1	A 1 1 m
83	8:c2	B 1 1 m
84	8:c3	I 1 1 m
85	9:c1	A 1 1 a
86	9:c2	B 1 1 n
87	9:c3	I 1 1 b
88	9:-c1	B 1 1 b
89	9:-c2	A 1 1 n
90	9:-c3	I 1 1 a
91	10:c	P 1 1 2/m
92	11:c	P 1 1 21/m
93	12:c1	A 1 1 2/m
94	12:c2	B 1 1 2/m
95	12:c3	I 1 1 2/m
96	13:c1	P 1 1 2/a
97	13:c2	P 1 1 2/n
98	13:c3	P 1 1 2/b
99	14:c1	P 1 1 21/a
100	14:c2	P 1 1 21/n
101	14:c3	P 1 1 21/b
102	15:c1	A 1 1 2/a
103	15:c2	B 1 1 2/n
104	15:c3	I 1 1 2/b
105	15:-c1	B 1 1 2/b
106	15:-c2	A 1 1 2/n
107	15:-c3	I 1 1 2/a
108	16	P 2 2 2
109	17	P 2 2 21
110	17:cab	P 21 2 2
111	17:bca	P 2 21 2
112	18	P 21 21 2
113	18:cab	P 2 21 21
114	18:bca	P 21 2 21

115	19	P 21 21 21
116	20	C 2 2 21
117	20:cab	A 21 2 2
118	20:bca	B 2 21 2
119	21	C 2 2 2
120	21:cab	A 2 2 2
121	21:bca	B 2 2 2
122	22	F 2 2 2
123	23	I 2 2 2
124	24	I 21 21 21
125	25	P m m 2
126	25:cab	P 2 m m
127	25:bca	P m 2 m
128	26	P m c 21
129	26:ba-c	P c m 21
130	26:cab	P 21 m a
131	26:-cba	P 21 a m
132	26:bca	P b 21 m
133	26:a-cb	P m 21 b
134	27	P c c 2
135	27:cab	P 2 a a
136	27:bca	P b 2 b
137	28	P m a 2
137	28:ba-c	P b m 2
139	28:cab	P 2 m b
140	28:-cba	P 2 c m
141	28:bca	P c 2 m
142	28:a-cb	P m 2 a
143	29	P c a 21
144	29:ba-c	P b c 21
145	29:cab	P 21 a b
146	29:-cba	P 21 c a
147	29:bca	P c 21 b
148	29:a-cb	P b 21 a
149	30	P n c 2
150	30:ba-c	P c n 2
151	30:cab	P 2 n a
152	30:-cba	P 2 a n
153	30:bca	P b 2 n
154	30:a-cb	P n 2 b
155	31	P m n 21

156	31:ba-c	P n m 21
157	31:cab	P 21 m n
158	31:-cba	P 21 n m
159	31:bca	P n 21 m
160	31:a-cb	P m 21 n
161	32	P b a 2
162	32:cab	P 2 c b
163	32:bca	P c 2 a
164	33	P n a 21
165	33:ba-c	P b n 21
166	33:cab	P 21 n b
167	33:-cba	P 21 c n
168	33:bca	P c 21 n
169	33:a-cb	P n 21 a
170	34	P n n 2
171	34:cab	P 2 n n
172	34:bca	P n 2 n
173	35	C m m 2
174	35:cab	A 2 m m
175	35:bca	B m 2 m
176	36	C m c 21
177	36:ba-c	C c m 21
178	36:cab	A 21 m a
179	36:-cba	A 21 a m
180	36:bca	B b 21 m
181	36:a-cb	B m 21 b
182	37	C c c 2
183	37:cab	A 2 a a
184	37:bca	B b 2 b
185	38	A m m 2
186	38:ba-c	B m m 2
187	38:cab	B 2 m m
188	38:-cba	C 2 m m
189	38:bca	C m 2 m
190	38:a-cb	A m 2 m
191	39	A b m 2
192	39:ba-c	B m a 2
193	39:cab	B 2 c m
194	39:-cba	C 2 m b
195	39:bca	C m 2 a
196	39:a-cb	A c 2 m

197	40	A m a 2
198	40:ba-c	B b m 2
199	40:cab	B 2 m b
200	40:-cba	C 2 c m
201	40:bca	C c 2 m
202	40:a-cb	A m 2 a
203	41	A b a 2
204	41:ba-c	B b a 2
205	41:cab	B 2 c b
206	41:-cba	C 2 c b
207	41:bca	C c 2 a
208	41:a-cb	A c 2 a
209	42	F m m 2
210	42:cab	F 2 m m
211	42:bca	F m 2 m
212	43	F d d 2
213	43:cab	F 2 d d
214	43:bca	F d 2 d
215	44	I m m 2
216	44:cab	I 2 m m
217	44:bca	I m 2 m
218	45	I b a 2
219	45:cab	I 2 c b
220	45:bca	I c 2 a
221	46	I m a 2
222	46:ba-c	I b m 2
223	46:cab	I 2 m b
224	46:-cba	I 2 c m
225	46:bca	I c 2 m
226	46:a-cb	I m 2 a
227	47	P m m m
228	48:1	P n n n:1
229	48:2	P n n n:2
230	49	P c c m
231	49:cab	P m a a
232	49:bca	P b m b
233	50:1	P b a n:1
234	50:2	P b a n:2
235	50:1cab	P n c b:1
236	50:2cab	P n c b:2
237	50:1bca	P c n a:1

238	50:2bca	P c n a:2
239	51	P m m a
240	51:ba-c	P m m b
241	51:cab	P b m m
242	51:-cba	P c m m
243	51:bca	P m c m
244	51:a-cb	P m a m
245	52	P n n a
246	52:ba-c	P n n b
247	52:cab	P b n n
248	52:-cba	P c n n
249	52:bca	P n c n
250	52:a-cb	P n a n
251	53	P m n a
252	53:ba-c	P n m b
253	53:cab	P b m n
254	53:-cba	P c n m
255	53:bca	P n c m
256	53:a-cb	P m a n
257	54	P c c a
258	54:ba-c	P c c b
259	54:cab	P b a a
260	54:-cba	P c a a
261	54:bca	P b c b
262	54:a-cb	P b a b
263	55	P b a m
264	55:cab	P m c b
265	55:bca	P c m a
266	56	P c c n
267	56:cab	P n a a
268	56:bca	P b n b
269	57	P b c m
270	57:ba-c	P c a m
271	57:cab	P m c a
272	57:-cba	P m a b
273	57:bca	P b m a
274	57:a-cb	P c m b
275	58	P n n m
276	58:cab	P m n n
277	58:bca	P n m n
278	59:1	P m m n:1

279	59:2	P m m n:2
280	59:1cab	P n m m:1
281	59:2cab	P n m m:2
282	59:1bca	P m n m:1
283	59:2bca	P m n m:2
284	60	P b c n
285	60:ba-c	P c a n
286	60:cab	P n c a
287	60:-cba	P n a b
288	60:bca	P b n a
289	60:a-cb	P c n b
290	61	P b c a
291	61:ba-c	P c a b
292	62	P n m a
293	62:ba-c	P m n b
294	62:cab	P b n m
295	62:-cba	P c m n
296	62:bca	P m c n
297	62:a-cb	P n a m
298	63	C m c m
299	63:ba-c	C c m m
300	63:cab	A m m a
301	63:-cba	A m a m
302	63:bca	B b m m
303	63:a-cb	B m m b
304	64	C m c a
305	64:ba-c	C c m b
306	64:cab	A b m a
307	64:-cba	A c a m
308	64:bca	B b c m
309	64:a-cb	B m a b
310	65	C m m m
311	65:cab	A m m m
312	65:bca	B m m m
313	66	C c c m
314	66:cab	A m a a
315	66:bca	B b m b
316	67	C m m a
317	67:ba-c	C m m b
318	67:cab	A b m m
319	67:-cba	A c m m

320	67:bca	B m c m
321	67:a-cb	B m a m
322	68:1	C c c a:1
323	68:2	C c c a:2
324	68:1ba-c	C c c b:1
325	68:2ba-c	C c c b:2
326	68:1cab	A b a a:1
327	68:2cab	A b a a:2
328	68:1-cba	A c a a:1
329	68:2-cba	A c a a:2
330	68:1bca	B b c b:1
331	68:2bca	B b c b:2
332	68:1a-cb	B b a b:1
333	68:2a-cb	B b a b:2
334	69	F m m m
335	70:1	F d d d:1
336	70:2	F d d d:2
337	71	I m m m
338	72	I b a m
339	72:cab	I m c b
340	72:bca	I c m a
341	73	I b c a
342	73:ba-c	I c a b
343	74	I m m a
344	74:ba-c	I m m b
345	74:cab	I b m m
346	74:-cba	I c m m
347	74:bca	I m c m
348	74:a-cb	I m a m
349	75	P 4
350	76	P 41
351	77	P 42
352	78	P 43
353	79	I 4
354	80	I 41
355	81	P -4
356	82	I -4
357	83	P 4/m
358	84	P 42/m
359	85:1	P 4/n:1
360	85:2	P 4/n:2

361	86:1	P 42/n:1
362	86:2	P 42/n:2
363	87	I 4/m
364	88:1	I 41/a:1
365	88:2	I 41/a:2
366	89	P 4 2 2
367	90	P 42 1 2
368	91	P 41 2 2
369	92	P 41 21 2
370	93	P 42 2 2
371	94	P 42 21 2
372	95	P 43 2 2
373	96	P 43 21 2
374	97	I 4 2 2
375	98	I 41 2 2
376	99	P 4 m m
377	100	P 4 b m
378	101	P 42 c m
379	102	P 42 n m
380	103	P 4 c c
381	104	P 4 n c
382	105	P 42 m c
383	106	P 42 b c
384	107	I 4 m m
385	108	I 4 c m
386	109	I 41 m d
387	110	I 41 c d
388	111	P -4 2 m
389	112	P -4 2 c
390	113	P -4 21 m
391	114	P -4 21 c
392	115	P -4 m 2
393	116	P -4 c 2
394	117	P -4 b 2
395	118	P -4 n 2
396	119	I -4 m 2
397	120	I -4 c 2
398	121	I -4 2 m
399	122	I -4 2 d
400	123	P 4/m m m
401	124	P 4/m c c

402	125:1	P 4/n b m:1
403	125:2	P 4/n b m:2
404	126:1	P 4/n n c:1
405	126:2	P 4/n n c:2
406	127	P 4/m b m
407	128	P 4/m n c
408	129:1	P 4/n m m:1
409	129:2	P 4/n m m:2
410	130:1	P 4/n c c:1
411	130:2	P 4/n c c:2
412	131	P 42/m m c
413	132	P 42/m c m
414	133:1	P 42/n b c:1
415	133:2	P 42/n b c:2
416	134:1	P 42/n n m:1
417	134:2	P 42/n n m:2
418	135	P 42/m b c
419	136	P 42/m n m
420	137:1	P 42/n m c:1
421	137:2	P 42/n m c:2
422	138:1	P 42/n c m:1
423	138:2	P 42/n c m:2
424	139	I 4/m m m
425	140	I 4/m c m
426	141:1	I 41/a m d:1
427	141:2	I 41/a m d:2
428	142:1	I 41/a c d:1
429	142:2	I 41/a c d:2
430	143	P 3
431	144	P 31
432	145	P 32
433	146:H	R 3:H
434	147	P -3
435	148:H	R -3:H
436	149	P 3 1 2
437	150	P 3 2 1
438	151	P 31 1 2
439	152	P 31 2 1
440	153	P 32 1 2
441	154	P 32 2 1
442	155:H	R 32:H

443	156	P 3 m 1
444	157	P 3 1 m
445	158	P 3 c 1
446	159	P 3 1 c
447	160:H	R 3 m:H
448	161:H	R 3 c:H
449	162	P -3 1 m
450	163	P -3 1 c
451	164	P -3 m 1
452	165	P -3 c 1
453	166:H	R -3 m:H
454	167:H	R -3 c:H
455	146:R	R 3:R
456	148:R	R -3:R
457	155:R	R 32:R
458	160:R	R 3 m:R
459	161:R	R 3 c:R
460	166:R	R -3 m:R
461	167:R	R -3 c:R
462	168	P 6
463	169	P 61
464	170	P 65
465	171	P 62
466	172	P 64
467	173	P 63
468	174	P -6
469	175	P 6/m
470	176	P 63/m
471	177	P 6 2 2
472	178	P 61 2 2
473	179	P 65 2 2
474	180	P 62 2 2
475	181	P 64 2 2
476	182	P 63 2 2
477	183	P 6 m m
478	184	P 6 c c
479	185	P 63 c m
480	186	P 63 m c
481	187	P -6 m 2
482	188	P -6 c 2
482	189	P -6 2 m

484	190	P -6 2 c
485	191	P 6/m m m
486	192	P 6/m c c
487	193	P 63/m c m
488	194	P 63/m m c
489	195	P 2 3
490	196	F 2 3
491	197	I 2 3
492	198	P 21 3
493	199	I 21 3
494	200	P m -3
495	201:1	P n -3:1
496	201:2	P n -3:2
497	202	F m -3
498	203:1	F d -3:1
499	203:2	F d -3:2
500	204	I m -3
501	205	P a -3
502	206	I a -3
503	207	P 4 3 2
504	208	P 42 3 2
505	209	F 4 3 2
506	210	F 41 3 2
507	211	I 4 3 2
508	212	P 43 3 2
509	213	P 41 3 2
510	214	I 41 3 2
511	215	P -4 3 m
512	216	F -4 3 m
513	217	I -4 3 m
514	218	P -4 3 n
515	219	F -4 3 c
516	220	I -4 3 d
517	221	P m -3 m
518	222:1	P n -3 n:1
519	222:2	P n -3 n:2
520	223	P m -3 n
521	224:1	P n -3 m:1
522	224:2	P n -3 m:2
523	225	F m -3 m
524	226	F m -3 c

525	227:1	F d -3 m:1
526	227:2	F d -3 m:2
527	228:1	F d -3 c:1
528	228:2	F d -3 c:2
529	229	I m -3 m
530	230	I a -3 d

14.5 Appendix E: Extinction Symbols and their Space Groups

In order to use this table look up the extinction symbol returned by the space group determination program, in the left-hand column of the table. The possible space groups for that extinction symbol are then listed in the right-hand columns.

Monoclinic a axis			
P - 1 1	P 2 1 1	P m 1 1	P 2/m 1 1
P 21 1 1	P 21 1 1		P 21/m 1 1
P b 1 1		P b 1 1	P 2/b 1 1
P 21/b 1 1			P 21/b 1 1
P c 1 1		P c 1 1	P 2/c 1 1
P 21/c 1 1			P 21/c 1 1
P n 1 1		P n 1 1	P 2/n 1 1
P 21/n 1 1			P 21/n 1 1
C - 1 1	C 2 1 1	C m 1 1	C 2/m 1 1
C n 1 1		C n 1 1	C 2/n 1 1
B - 1 1	B 2 1 1	B m 1 1	B 2/m 1 1
B b 1 1		B b 1 1	B 2/b 1 1
I - 1 1	I 2 1 1	I m 1 1	I 2/m 1 1
I c 1 1		I c 1 1	I 2/c 1 1
Monoclinic b axis			
P 1 - 1	P 1 2 1	P 1 m 1	P 1 2/m 1
P 1 21 1	P 1 21 1		P 1 21/m 1
P 1 a 1		P 1 a 1	P 1 2/a 1
P 1 21/a 1			P 1 21/a 1
P 1 c 1		P 1 c 1	P 1 2/c 1
P 1 21/c 1			P 1 21/c 1
P 1 n 1		P 1 n 1	P 1 2/n 1
P 1 21/n 1			P 1 21/n 1
C 1 - 1	C 1 2 1	C 1 m 1	C 1 2/m 1
C 1 c 1		C 1 c 1	C 1 2/c 1
A 1 - 1	A 1 2 1	A 1 m 1	A 1 2/m 1
A 1 n 1		A 1 n 1	A 1 2/n 1
I 1 - 1	I 1 2 1	I 1 m 1	I 1 2/m 1

I 1 a 1		I 1 a 1	I 1 2/a 1
Monoclinic c axis			
P 1 1 -	P 1 1 2	P 1 1 m	P 1 1 2/m
P 1 1 21	P 1 1 21		P 1 1 21/m
P 1 1 a		P 1 1 a	P 1 1 2/a
P 1 1 21/a			P 1 1 21/a
P 1 1 b		P 1 1 b	P 1 1 2/b
P 1 1 21/c			P 1 1 21/b
P 1 1 n		P 1 1 n	P 1 1 2/n
P 1 1 21/n			P 1 1 21/n
B 1 1 -	B 1 1 2	B 1 1 m	B 1 1 2/m
B 1 1 n		B 1 1 n	B 1 1 2/n
A 1 1 -	A 1 1 2	A 1 1 m	A 1 1 2/m
A 1 1 a		A 1 1 a	A 1 1 2/a
I 1 1 -	I 1 1 2	I 1 1 m	I 1 1 2/m
I 1 1 b		I 1 1 b	I 1 1 2/b
Orthorhombic			
P - - -	P 2 2 2	P m m 2	P m m m
		P m 2 m	
		P 2 m m	
P - - 21	P 2 2 21		
P - 21 -	P 2 21 2		
P - 21 21	P 2 21 21		
P 21 - -	P 21 2 2		
P 21 - 21	P 21 2 21		
P 21 21 -	P 21 21 2		
P 21 21 21	P 21 21 21		
P - - a		P m 2 a	
		P 21 m a	P m m a
P - - b		P m 21 b	P m 21 b
		P 2 m b	P m m b
P - - n		P m 21 n	
		P 21 m n	P m m n
P - a -		P m a 2	P m a m
		P 21 a m	
P - a a		P 2 a a	P m a a
P - a b		P 21 a b	P m a b
P - a n		P 2 a n	P m a n
P - c -		P m c 21	
		P 2 c m	P m c m
P - c a		P 21 c a	P m c a

P - c b		P 2 c b	P m c b
P - c n		P 21 c n	P m c n
P - n -		P m n 21	
		P 21 n m	P m n m
P - n a		P 2 n a	P m n a
P - n b		P 21 n b	P m n b
P - n n		P 2 n n	P m n n
P b - -		P b m 2	
		P b 21 m	P b m m
P b - a		P b 21 a	P b m a
P b - b		P b 2 b	P b m b
P b - n		P b 2 n	P b m n
P b a -		P b a 2	P b a m
P b a a			P b a a
P b a b			P b a b
P b a n			P b a n
P b c -		P b c 21	P b c m
P b c a			P b c a
P b c b			P b c b
P b c n			P b c n
P b n -		P b n 21	P b n m
P b n a			P b n a
P b n b			P b n b
P b n n			P b n n
P c - -		P c m 21	
		P c 2 m	P c m m
P c - a		P c 2 a	P c m a
P c - b		P c 21 b	P c m b
P c - n		P c 21 n	P c m n
P c a -		P c a 21	P c a m
P c a a			P c a a
P c a b			P c a b
P c a n			P c a n
P c c -		P c c 2	P c c m
P c c a			P c c a
P c c b			P c c b
P c c n			P c c n
P c n -		P c n 2	P c n m
P c n a			P c n a
P c n b			P c n b
P c c n			P c c n

P n - -		P n m 21	P n m m
			P n 21 m
P n - a		P n 21 a	P n m a
P n - b		P n 2 b	P n m b
P n - n		P n 2 n	P n m n
P n a -		P n a 21	P n a m
P n a a			P n a a
P n a b			P n a b
P n a n			P n a n
P n c -		P n c 2	P n c m
P n c a			P n c a
P n c b			P n c b
P n c n			P n c n
P n n -		P n n 2	P n n m
P n n a			P n n a
P n n b			P n n b
P n n n			P n n n
C - - -	C 2 2 2	C m m 2	C m m m
		C m 2 m	
		C 2 m m	
C - - 21	C 2 2 21		
C - - (ab)		C m 2 a	C m m a
		C 2 m b	C m m b
C - c -		C m c 21	C m c m
		C 2 c m	
C - c (ab)		C 2 c b	C m c a
C c - -		C c m 21	C c m m
		C c 2 m	
C c - (ab)		C c 2 a	C c m b
C c c -		C c c 2	C c c m
C c c (ab)			C c c a
			C c c b
B - - -	B 2 2 2	B m m 2	B m m m
		B m 2 m	
		B 2 m m	
B - 21 -	B 2 21 2		
B - - b		B m 21 b	B m m b
		B 2 m b	
B - (ac)-		B m a 2	B m a m
		B 2 c m	B m c m
B - (ac)b		B 2 c b	B m a b

B b - -		B b m 2	B b m m
		B b 21 m	
B b - b		B b 2 b	B b m b
B b (ac)-		B b a 2	B b c m
B b (ac)b			B b a b
			B b a c
A - - -	A 2 2 2	A m m 2	A m m m
		A m 2 m	
		A 2 2 m	
A 21 - -	A 21 2 2		
A - - a		A m 2 a	A m m a
		A 21 m a	
A - a -		A m a 2	A m a m
		A 21 a m	
A - a a		A 2 a a	A m a a
A(bc)- -		A b m 2	A b m m
		A c 2 m	A c m m
A(bc)- a		A c 2 a	A b m a
A(bc)a -		A b a 2	A c a m
A(bc)a a			A b a a
			A ca a
I - - -	I 2 2 2	I m m 2	I m m m
		I m 2 m	
		I 2 2 m	
I - - (ab)		I m 2 a	I m m a
		I m 2 b	I m m b
I - (ac)-		I m a 2	I m a m
		I 2 c m	I m c m
I - c b		I 2 c b	I m c b
I(bc)- -		I b m 2	I b m m
		I c m 2	I c m m
I c - a		I c 2 a	I c m a
I b a -		I b a 2	I b a m
I b c a			I b c a
F _ _ _	F 2 2 2	F m m 2	F m m m
		F m 2 m	
		F 2 m m	
F _ d d		F 2 d d	
F d _ d		F d 2 d	
F d d _		F d d 2	
F d d d		F d d d	

Tetragonal								
P - - -	P4	P-4	P4/m	P 4 2 2	P 4 m m	P -4 2 m	P4/m m m	
					P -4 m 2			
P - 21 -				P 4 21 2	P -4 21 m			
P 42 - -	P42		P42/m	P 42 2 2				
P 42 21 -				P 42 21 2				
P 41 - -	P41			P 41 2 2				
	P43			P 43 2 2				
P 41 21 _				P 41 21 2				
				P 43 21 2				
P - - c					P 42 m c	P -4 2 c	P42/m m c	
P - 21 c						P -4 21 c		
P - b -					P 4 b m	P -4 b 2	P4/m b m	
P - b c					P 42 b c		P42/m b c	
P - c -					P 42 c m	P -4 c 2	P42/m c m	
P - c c					P 4 c c		P4/m c c	
P - n -					P 42 n m	P -4 n 2	P42/m n m	
P - n c					P 4 n c		P4/ m n c	
P n - -			P4/n				P4/n m m	
P 42/n - -			P42/n					
P n - c							P42/n m c	
P n b -							P4/ n b m	
P n b c							P42/n b c	
P n c -							P42/n c m	
P n c c							P4/n c c	
P n n -							P42/n n m	
P n n c							P4/n n c	
I - - -	I4	I-4	I4/m	I 4 2 2	I 4 m m	I -4 2 m	I4/m m m	
						I -4 m 2		
I 41 - -	I41			I41 2 2				
I - - d					I 41 m d	I -4 2 d		
I - c -					I 4 c m	I -4 c 2	I4/m c m	
I - c d						I 41 c d		
I 41/a - -			I41/a					
I a - d							I41/a m d	
I a c d							I41/a c d	
Trigonal								

P - - -	P3	P-3	P3 2 1		P3 m 1		P-3 m 1	P3 1 2
					P3 1 m		P-3 1 m	
P 31 - -	P31		P31 2 1					
			P31 1 2					
	P32		P32 2 1					
			P32 1 2					
P - - c					P 3 1 c		P-3 1 c	
P - c -					P 3 c 1		P-3 c 1	
R - - -	R3	R-3	R32	R3m		R-3m		
R - - c				R3c		R-3c		
Hexagonal								
P - - -	P6	P-6	P6/m	P 6 2 2		P 6 m m	P-6 2 m	P6/mmm
							P-6 m 2	
P 63 - -	P63		P63/m	P63 2 2				
P 62 - -	P62			P62 2 2				
	P64			P64 2 2				
P 61 - -	P61			P61 2 2				
	P65			P65 2 2				
P - - c						P 63 m c	P-6 2 c	P63/mmc
P - c -						P 63 c m	P-6 c 2	P63/mcm
P - c c						P 6 c c		P6/mcc
Cubic								
P - - -	P23	Pm-3	P4 3 2		P-4 3 m		P m -3 m	
P 21(42) - -		P21 3	P42 3 2					
P 41 - -			P41 3 2					
			P43 3 2					
P - - n					P-4 3 n		P m -3 n	
P a - -		Pa-3						
P n - -		Pn-3					P n -3 m	
P n - n							P n -3 n	
I - - -	I23	Im-3	I4 3 2		I-4 3 2		I m -3 m	
	I21 3							
I 41 - -			I41 3 2					
I - - d					I-4 3 d			
I a - -		Ia-3						
I a - d							I a -3 d	
F - - -	F23	Fm-3	F4 3 2		F-4 3 m		F m -3 m	
F 41 - -			F41 3 2					
F - - c					F-4 3 c		F m -3 c	

F d - -		Fd-3					F d -3 m	
F d - c							F d -3 c	

14.6 Appendix F: Using the Cambridge Structural Database

The Cambridge Structural Database (CSD) contains >430,000 crystal structures for organic and organometallic molecules. The CSD is part of the CSD System which also includes software for:

- Search, retrieval and analysis of structures - *ConQuest*
- Crystal structure visualisation - *Mercury*
- Generation of in-house databases searchable alongside the CSD - *PreQuest*
- Data analysis - *Vista*

The CSD System also incorporates *IsoStar*, a library of intermolecular interactions, containing data derived from both the CSD and PDB, and *Mogul*, a molecular geometry library.

For more information about the Cambridge Crystallographic Data Centre (CCDC) see:
http://www. ccdc.cam.ac.uk

14.6.1 F.1 Source of Geometry for Molecular Models

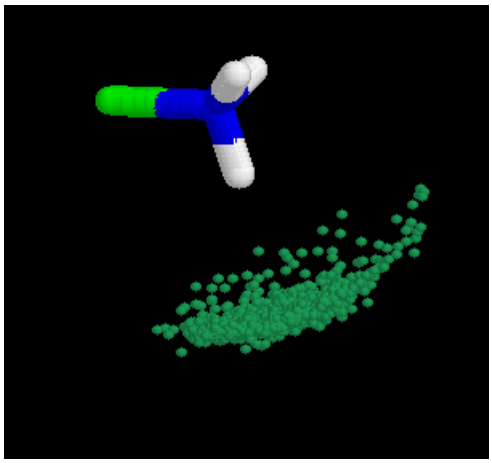
- The search program *ConQuest* allows one to retrieve all molecular fragments specified by drawing chemical diagrams. The chemical environment of the fragments can be specified very precisely using a variety of attributes such as number of hydrogens per atom, cyclicity of bonds, etc. The geometry of these fragments may be saved as a list of geometric parameters defined by the user, and displayed as histograms and scattergrams by the program *Vista*.
- DASH provides a direct link to *Mogul*, a molecular geometry database which forms part of the *CSD System* and is available from the *CCDC*. Searches for molecular fragments, bond lengths, bond angles and torsion angles can be performed quickly and easily.

14.6.2 F.2 Checking of Best Solutions against CSD Packing Motif

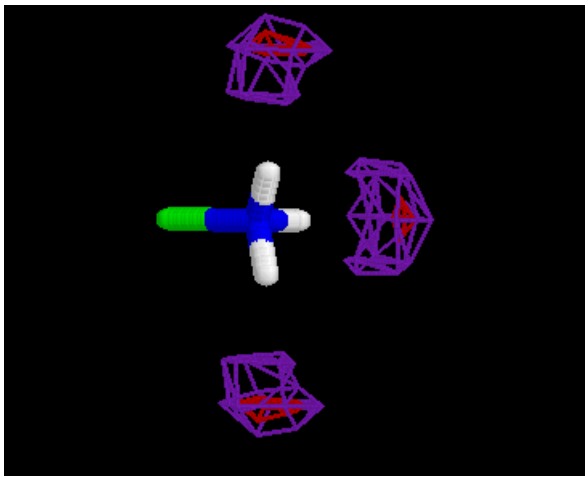
- The *ConQuest* program is also able to search for intermolecular interactions, and store parameters in the same way as for intramolecular geometry, for examination by *Vista*, a statistical analysis package. Examples of such useful information are H-bonds and chloride ion interaction with charged nitrogen. The packing motifs of the retrieved structures can be examined using the visualiser *Mercury* or *Pluto*. These programs are particularly useful for easy exploration of H-bonding motifs.
- The *IsoStar* library of intermolecular interactions is also provided with the CSD system. This is an extensive library of scattergrams of the intermolecular crystal environment of a set of well-known chemical groups. Each group (termed the central group) has a set of pre-processed

scattergrams of interacting groups in the CSD, taking account of symmetry to produce an overall picture. These scattergrams can be easily inspected using the Rasmol visualiser provided.

An IsoStar example is shown here of a central charged amine group, NH_3^+, approached by a chloride ion, Cl^-.

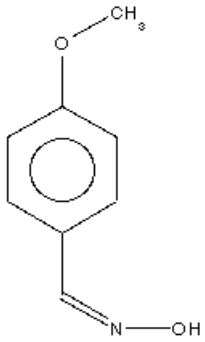


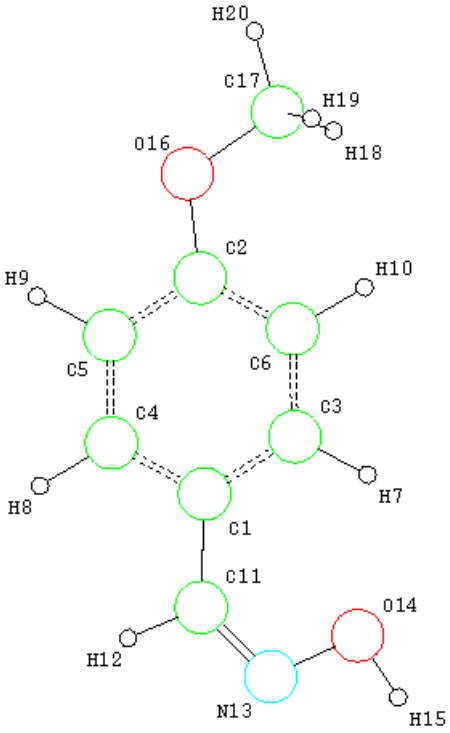
In the IsoStar contour view option, the scatterplot has been contoured to show the preferred positions of the chloride at about 3.0 Å from the nitrogen.

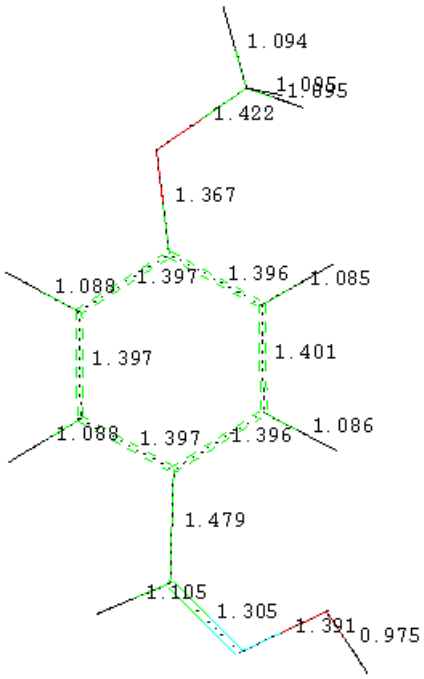


14.7 Appendix G: Z-matrix format

Example molecule, Z-4-methoxybenzaldoxime, CSD reference code AANHOX:
There are two variable torsion angles about bonds C2-O16 and C1-C11.


CH3
O
N
OH


H20
C17
H19
H18
O16
C2
H9
H10
C5
C6
C4
C3
H8
H7
C1
C11
O14
H12
N13
H15



EXAMPLE MOL2 file

```
#
#     File Created by: PC Spartan Pro Export
#

@<TRIPOS>MOLECULE
C:\motherwell\samoxime.mo2
20 20
SMALL
NO_CHARGES


@<TRIPOS>ATOM
    1   C1         0.293217313     0.250035865     1.069792204    C.ar   1  Molecule001
    2   C2        -0.008979730     0.027063995     3.852291799    C.ar   1  Molecule001
    3   C3         0.488999937    -0.978777859     1.703096616    C.ar   1  Molecule001
    4   C4        -0.080645376     1.364432686     1.825562143    C.ar   1  Molecule001
    5   C5        -0.223538726     1.250142376     3.211062816    C.ar   1  Molecule001
    6   C6         0.344323476    -1.088558606     3.092210833    C.ar   1  Molecule001
    7   H7         0.737253557    -1.865934089     1.127281963       H   1  Molecule001
    8   H8        -0.252034022     2.327749313     1.352239204       H   1  Molecule001
    9   H9        -0.504578299     2.121804863     3.797847048       H   1  Molecule001
   10   H10        0.508767609    -2.061069866     3.544242324       H   1  Molecule001
   11   C11        0.389641286     0.420855758    -0.395989867     C.2   1  Molecule001
   12   H12       -0.290524363     1.180800541    -0.819438688       H   1  Molecule001
   13   N13        1.166972637    -0.174090243    -1.259879230     N.2   1  Molecule001
   14   O14        2.081028705    -1.042636861    -0.672914357     O.3   1  Molecule001
   15   H15        2.599746303    -1.348446047    -1.440188292       H   1  Molecule001
   16   O16       -0.183225243     0.055644157     5.208123004     O.3   1  Molecule001
   17   C17        0.050187143    -1.157264365     5.913234641     C.3   1  Molecule001
```

```
   18   H18        1.085947683    -1.491802143     5.793237167       H   1  Molecule001
   19   H19       -0.655864517    -1.935183860     5.604477331       H   1  Molecule001
   20   H20       -0.114861688    -0.961941388     6.977167021       H   1  Molecule001


@<TRIPOS>BOND
    1      1      4    ar
    2      4      5    ar
    3      2      5    ar
    4      2      6    ar
    5      3      6    ar
    6      1      3    ar
    7      5      9    1
    8      6     10    1
    9      3      7    1
   10      4      8    1
   11     11     12    1
   12      1     11    1
   13     11     13    2
   14     14     15    1
   15     13     14    1
   16      2     16    1
   17     17     18    1
   18     17     19    1
   19     17     20    1
   20     16     17    1


Z-Matrix file created by DASH from the input MOL2 file

Zmatrix generated by Mercury
1.0 1.0 1.0 90.0 90.0 90.0
  20   0
  C      0.0000000  0    0.0000000  0    0.0000000  0    0    0    0    0  3.0  1.0    3 C3
  C      1.3962050  0    0.0000000  0    0.0000000  0    1    0    0    0  3.0  1.0    1 C1 C3
  C      1.4009359  0  120.2814856  0    0.0000000  0    1    2    0    0  3.0  1.0    6 C6 C3 C1
  H      1.0863878  0  120.6861954  0  178.8814973  0    1    2    3    0  6.0  1.0    7 H7 C3 C1 C6
  C      1.3974412  0  119.6071742  0   -1.5745262  0    2    1    3    0  3.0  1.0    4 C4 C1 C3 C6
  C      1.4788489  0  122.8266674  0 -176.4571818  0    2    1    5    0  3.0  1.0   11 C11 C1 C3 C4
  C      1.3954068  0  120.2386237  0    0.6027188  0    3    1    2    0  3.0  1.0    2 C2 C6 C3 C1
  H      1.0849666  0  117.8853515  0  179.6006996  0    3    1    7    0  6.0  1.0   10 H10 C6 C3 C2
  C      1.3975310  0  119.8880618  0    1.5806770  0    5    2    1    0  3.0  1.0    5 C5 C4 C1 C3
  H      1.0869166  0  120.8934394  0  179.2769860  0    5    2    9    0  6.0  1.0    8 H8 C4 C1 C5
  H      1.1042872  0  114.7967855  0  146.8411988  1    6    2    1    0  6.0  1.0   12 H12 C11 C1 C3
  N      1.3055687  0  129.9435928  0  178.8979722  0    6    2   11    0  3.0  1.0   13 N13 C11 C1 H12
  O      1.3672807  0  126.0945149  0  179.9304618  0    7    3    1    0  3.0  1.0   16 O16 C2 C6 C3
  H      1.0877015  0  119.7165368  0  179.3664126  0    9    5    2    0  6.0  1.0    9 H9 C5 C4 C1
  O      1.3908269  0  113.3670309  0   -3.8330576  0   12    6    2    0  3.0  1.0   14 O14 N13 C11 C1
  C      1.4222555  0  116.9284636  0    1.6874795  1   13    7    3    0  3.0  1.0   17 C17 O16 C2 C6
  H      0.9753443  0  102.3173121  0 -176.8034053  0   15   12    6    0  6.0  1.0   15 H15 O14 N13 C11
  H      1.0950410  0  111.1884452  0   61.2946259  0   16   13    7    0  6.0  1.0   18 H18 C17 O16 C2
  H      1.0949880  0  111.1155213  0 -124.4607417  0   16   13   18    0  6.0  1.0   19 H19 C17 O16 H18
  H      1.0942323  0  107.7617586  0  117.7913565  0   16   13   18    0  6.0  1.0   20 H20 C17 O16 H18
```

The Z-Matrix format is commonly used in molecular modelling (see Appendix H: References, page 189). In this format we describe the molecular co-ordinates in terms of internal co-ordinates, namely the bond-lengths, bond-angles, and torsion angles. The molecule is assembled by building up the molecule atom-by-atom, and placing each atom at a given bond-length, bond angle and torsion angle with reference to the earlier atoms. Each atom-line effectively gives the “instructions” for adding another atom to the molecule. The atoms in this Z-matrix file are referred to by their sequence

numbers, I, J, K, L in this list. In the example above there are 20 atoms, which is specified as the first item on line 2 of the file, NAT. (Note that the atoms are presented in a different order from the input Cartesian co-ordinate file in mol2 or pdb format).

Line 1 Title line
Line 2 Is ignored
Line 3 NAT = the number of atoms following in the atom-list
IAT = the atom number to use as origin for DASH rotation parameters
= 0 is taken as using centre of mass as origin

Line 4 Atom-lines. These atoms are given sequence number I = 1 to NAT

Item 1 EL Element type
Item 2 BL Bond length in Angstroms to connect this atom to atom J (item 8 on line)
Item 3 FB Flag to signal fixed (=0) or variable (=1)
Item 4 BA Bond angle in degrees for angle I-J-K, where K is item 9 on line
Item 5 FA Flag to signal bond angle fixed/variable
Item 6 BT Torsion angle I-J-K-L, where atom L is item 10 on line
Item 7 FT Flag to signal torsion angle fixed/variable
Item 8 J atom number for bond I-J
Item 9 K atom number for angle I-J-K
Item 10 L atom number for torsion angle I-J-K-L
Item 11 Temperature factor to be used in DASH structure factor calculation
Item 12 Occupancy factor of atom, in range 0.0 to 1.0
Item 13 Original number
Item 14 Label of atom I in input Cartesian file
Item 15 Label of atom J in input Cartesian file
Item 16 Label of atom K in input Cartesian file
Item 17 Label of atom L in input Cartesian file

Notes:

- The flags FT for torsion angles are set as fixed or variable by the Z-Matrix generation program, using the chemical knowledge of the bond type and hybridisation state. It is possible to over-ride these settings, by locating the torsion angle with the help of the input atom labels e.g. O14-C11-N13-C1. There may be a case where one wishes to make this a variable torsion angle, although described as a double bond, by setting FT=1 and thus effectively allowing exploration of cis- and trans-isomers in the search.
- Only those torsion angles flagged with FT=1 are displayed as parameters in the *Simulated annealing parameter setup* window (see Section 10.3.6, page 109).

14.8 Appendix H: References

Original authors of the DASH program, before CCDC took over onward development and distribution of the program:
David, W. I. F, Shankland, K.

Background estimation using a robust Bayesian Analysis
David, W.I.F.D. & Sivia, D.S. (2001). *J. Appl. Cryst.,* **34**, 318-324

Correlated integrated intensities (GA based search):
Shankland, K., David, W.I.F. and Csoka, T. (1997) *Z Kristallogr.*, **212**, 550-552

Correlated integrated intensities (SA based search i.e. DASH):
David , W. I. F, Shankland, K. and Shankland N. (1998) *J. Chem. Soc. Chem.Commun.*, 931-932.

Extinction Symbol Program:
Markvardsen, A.J., David, W.I.F., Johnson, J.C., Shankland, K. (2001) *Acta Cryst.*, **A57**, 47-54

Large pharmaceutical molecule structure solution (telmisartan, two polymorphs):
Dinnebier, R.E., Wagner, M., Peters, F., Shankland, K. and David, W.I.F. (2000) *Z. Anorganische Und Allgemeine Chemie*, **626**, 1400-1405

Mercury: visualisation and analysis of crystal structures:
Macrae, C.F., Edgington, P.R., McCabe, P., Pidcock, E., Shields, G.P., Taylor, R., Towler, M., van de Streek, J. (2006) *J. Appl. Cryst.*, **39**, 453-457.

Mercury 2.0 - New features for the visualisation and investigation of crystal structures:
Macrae, C.F., Bruno, I.J., Chisholm, J.A., Edgington, P.R., McCabe, P., Pidcock, E., Rodriguez-Monge, L., Taylor, R., van de Streek, J., Wood, P.A. (2008) *J. Appl. Cryst.*, (*in press*).

Pawley Refinement:
Pawley, G. S. (1981) *J. Appl. Cryst*., **14**, 357

Retrieval of Crystallographically-Derived Molecular Geometry Information
Bruno, I.J., Cole, J.C., Kessler, M., Luo, J., Motherwell, W.D.S., Purkis, L.H., Smith, B.R.,Taylor, R., Cooper, R.I., Harris, S.E., Orpen, A.G. (2004) *J. Chem. Inf. Comput. Sci.*, **44**, 2133-2144.

Rotation of molecules (Quaternions):
Leach, A.R., *Molecular Modelling: Principles and Applications* (1996), Longman, Harlow (ISBN 0582239338), 382-385

Simulated Annealing:
Press, W.H., Flannery, B.P., Teukolsky, S.A. and Vetterling, W.T. *Numerical Recipes* (1986) Cambridge University Press, Cambridge (ISBN 0521308119), 274-277 & 326-331

Simplex Method of Optimisation:
Press, W.H., Flannery, B.P., Teukolsky, S.A. and Vetterling, W.T. *Numerical Recipes* (1986) Cambridge University Press, Cambridge (ISBN 0521308119), 289-293.

Two molecules in the Asymmetric Unit:
Bell, A.M.T., Smith, J.N.B., Attfield, J.P., Rawson, J.M., Shankland, K. and David, W.I.F. (1999) *New Journal Of Chemistry*, **23**, 565-567

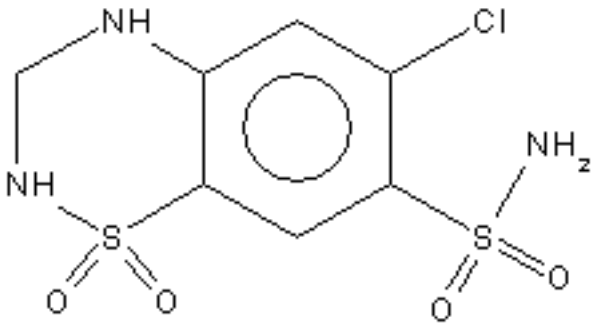
Z-matrix:
Leach, A.R., *Molecular Modelling: Principles and Applications* (1996), Longman (ISBN 0582239338), 1-3

15 TUTORIALS

15.1 Tutorial 1 - Step by Step Structure Solution of Hydrochlorothiazide

15.1.1 Introduction

The object of this tutorial is to guide you through the process of structure solution, using the molecule hydrochlorothiazide as an example. Tutorial 1 goes through the process in considerable detail; subsequent tutorial examples will be more concise, but will introduce other, new aspects of the structure solution process. This tutorial will take a novice user about 2 hours to complete and experienced powder crystallographers considerably less time.



15.1.2 Data

The data set *Tutorial_1.xye* is a synchrotron X-ray diffraction data set collected at 20 K on Beamline X7A of the Brookhaven National Synchrotron Light Source. The incident wavelength was 1.1294 Å and the sample was held in a 0.7 mm glass capillary.

15.1.3 Stage 1: Reading the data

Open DASH by double clicking on the DASH icon.

The DASH Wizard will guide you through the structure solution process, which is performed in a series of steps.

- Select **View data / determine peak positions** and click **Next >**.
- Click the **Browse...** button.
- Select the file *Tutorial_1.xye* (from, e.g. *C:\Program Files\CCDC\DASH 3.1\Documentation\Tutorial1\Data files)* and click **Open**. The diffraction data will be loaded into DASH. Click **Next >**.
- Check that the wavelength and radiation source have been set correctly and click **Next >**.

- Truncate the data to a resolution of 1.75Å and click **Next >**.
- The default window size setting of 100 should be good enough for this simple background. Click **Next >**.

15.1.4 Stage 2: Examining the Data

This section of the tutorial is purely descriptive so if you are familiar with handling powder diffraction data, skip to Stage 3.

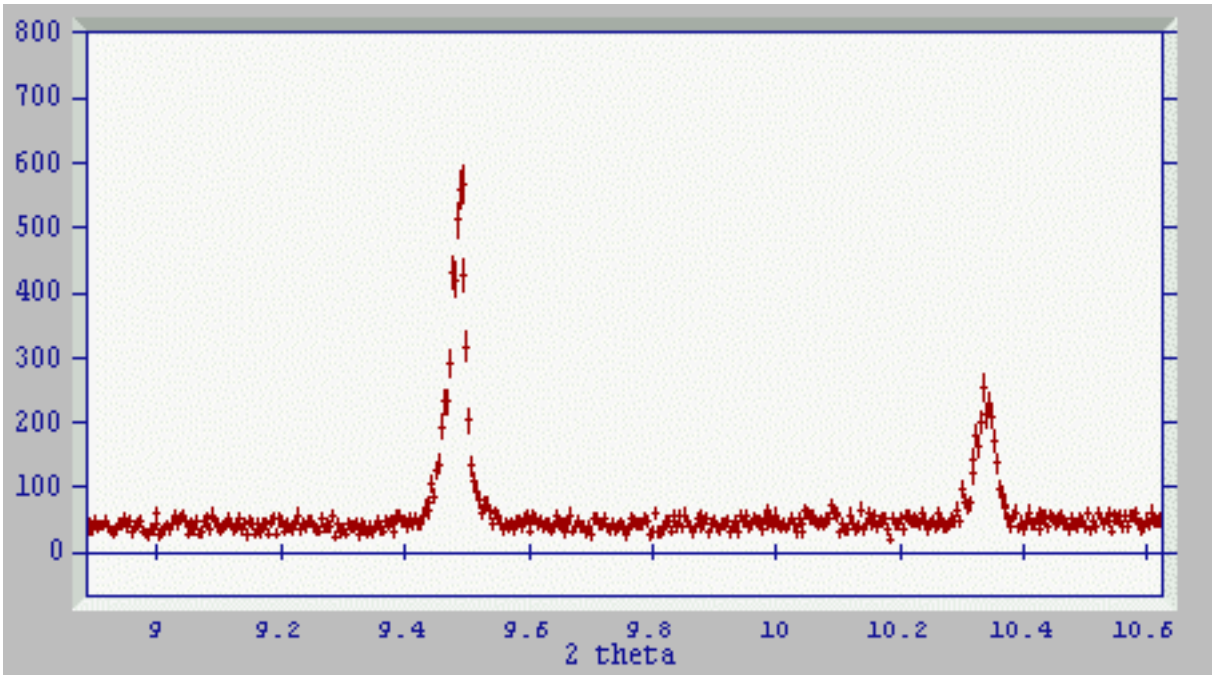
The data spans 5 to 44° 2q. If you open the file *Tutorial_1.xye* in an ASCII text editor such as Wordpad you'll see that the wavelength of 1.1294 is given on the first line.

The data consists of three columns.

```
1.1294
    5.000 81.96 10.952
    5.004 71.25 10.284
    ...
<bulk of data omitted for clarity>
    ...
    43.996 69.55 3.572
    44.000 68.28 3.540
```

Column 1 = 2q position
Column 2 = diffracted intensity (counts)
Column 3 = estimated standard deviation of the intensity

If you zoom in on the diffracted data as it is displayed in DASH, you will see that DASH displays both the intensity and the error bars. The simplest way to zoom is to use the left mouse button. Click and hold the left mouse button and drag out a rectangle around the area that you want to zoom in on. To zoom out, simply press the **Home** key on the keyboard (Note that there are other ways to zoom in on the data - see the DASH User Guide for details). Try zooming in on the two peaks that lie just either side of 10° 2q.



You can use the left and right cursor keys to move up and down the data in 2q. Some other useful keyboard shortcuts whilst examining data are:

Shift -å : Zoom in
Shift -é : Zoom out
Ctrl - å : Rescale the y-axis to the maximum in the current range

Whilst browsing the data, note the following features:

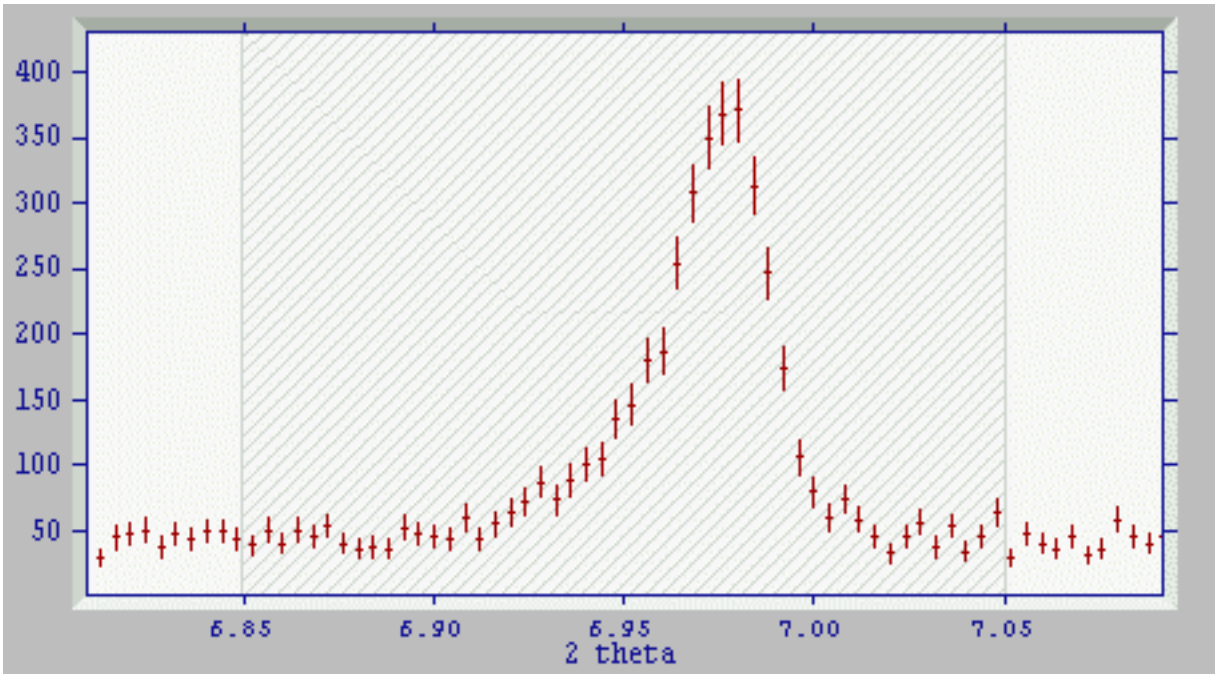
- The peak asymmetry (elongated tails to the left hand side of the peaks) in the low angle peaks, due to axial divergence.
- The flat background indicative of a lack of amorphous content.
- The sharp peaks, indicating a good crystalline sample.

- The excellent instrumental resolution. See, for example, the doublet of peaks around 12.17° 2q.
- The use of a small step size commensurate with the instrumental resolution and the narrow peaks, i.e. plenty of points across each peak.
- The fall off in diffracted intensity with increasing angle due to the Lorentz effect and thermal effects.
- The increasing number of peaks per unit angle with increasing angle.
- The excellent signal to noise ratio, even at the maximum diffraction angle, i.e. peaks can still be clearly discriminated from background.

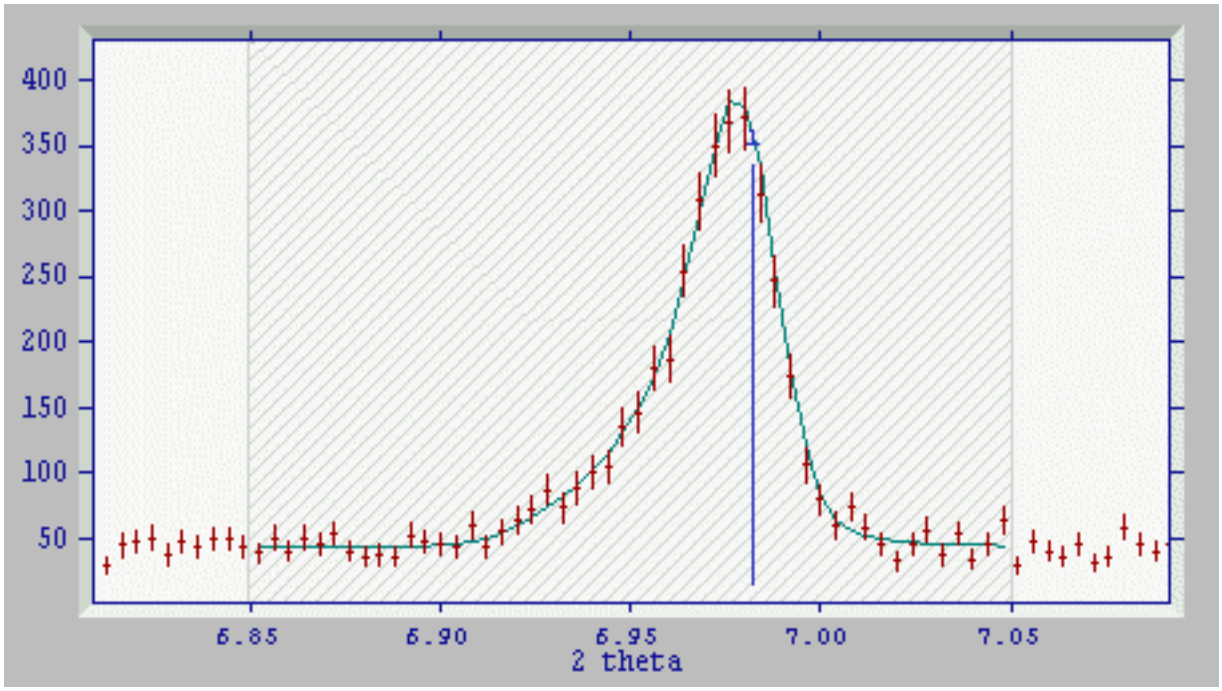
15.1.5 Stage 3. Fitting the Peaks to Determine the Exact Peak Positions

We need accurate estimates of the 2q positions of the first 20 or so peaks in the diffraction pattern in order to index the diffraction data, i.e. determine the unit cell and hence the Laue class of the crystal. DASH makes this process quick and easy by fitting entire peaks accurately. It is important to emphasise that we are only interested in peak positions, not peak intensities, at this stage, so weak peaks are every bit as important in indexing as strong ones. The first peak in the diffraction pattern is at just under 7°. To fit this peak:

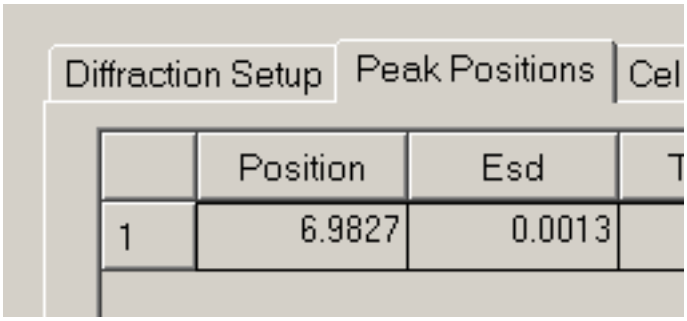
- Zoom in to the area around the peak.
- Sweep out an area using the right mouse button i.e. move to about 6.85° 2q, click the right-hand mouse button and hold down as you sweep right to about 7.05° before releasing the right button. The hatched area now covers the peak and enough background either side to allow an accurate estimate of the peak parameters. If you are not happy with the area swept out (e.g. if your finger slipped as you were sweeping), simply put the cursor inside the hatched area and press the **Delete** key on the keyboard to remove the current selected area, then try again.



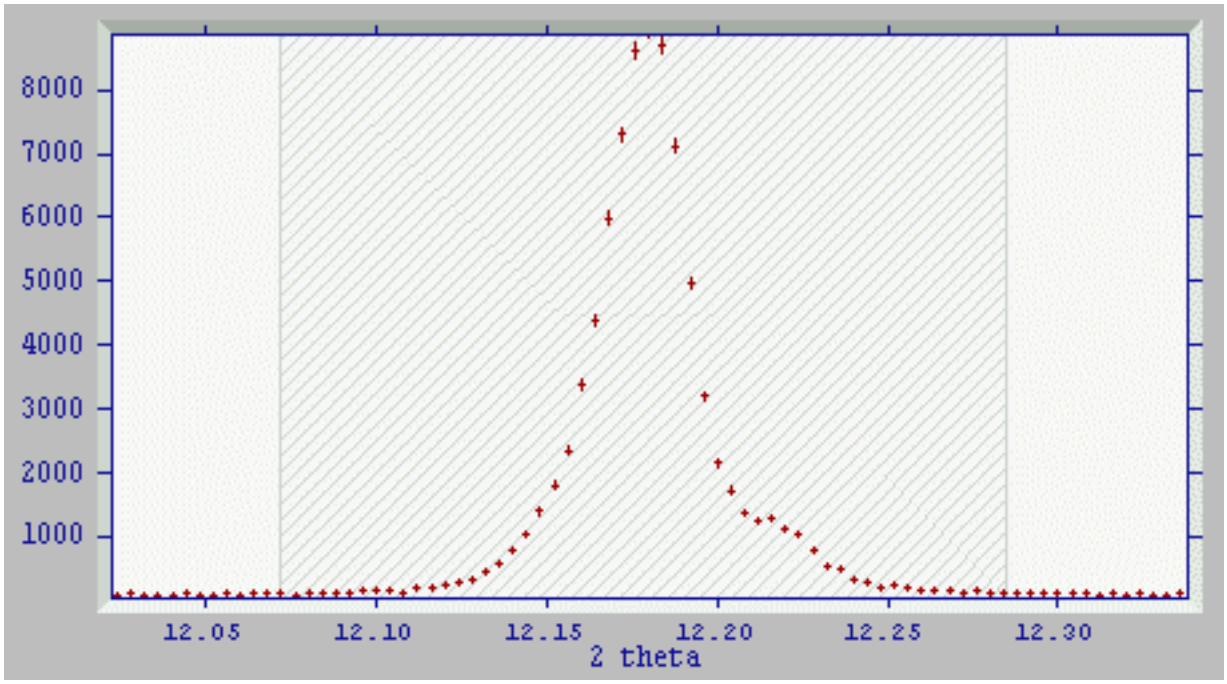
- With the cursor in the hatched area, press the **Return** or **Enter** key to fit the peak.



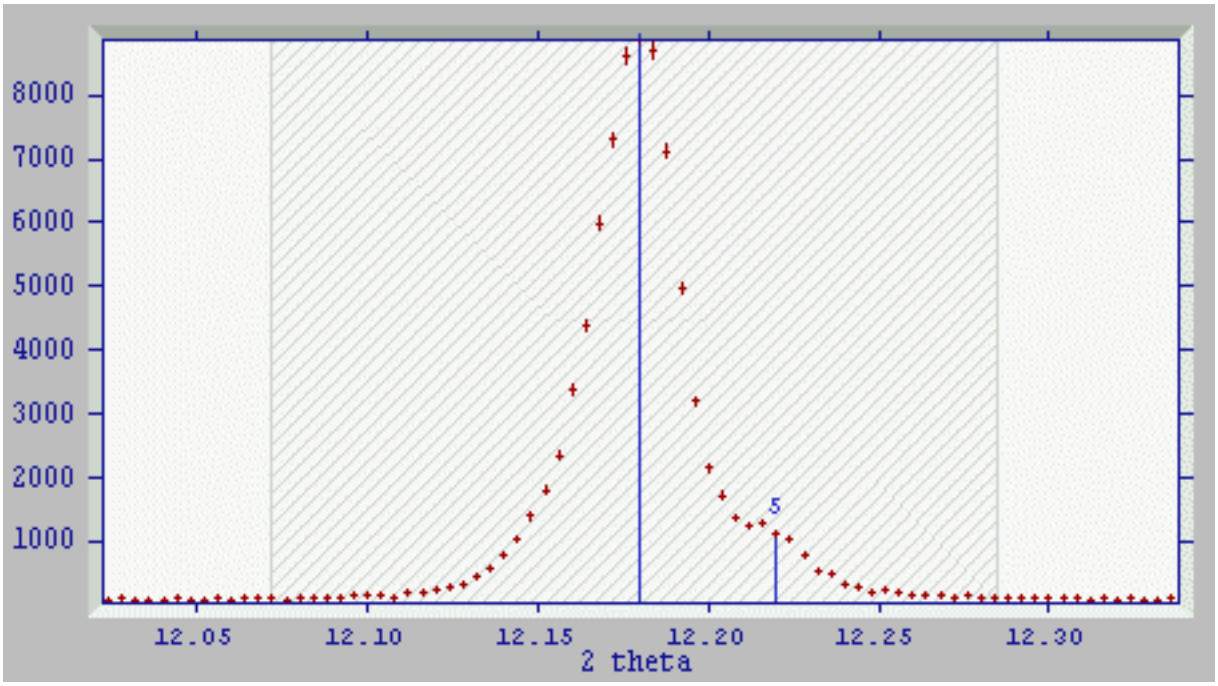
- The solid green line indicates the fit to the data, whilst the vertical blue line indicates the peak position. Selecting **Peak Positions** from the **View** menu shows the exact peak position:



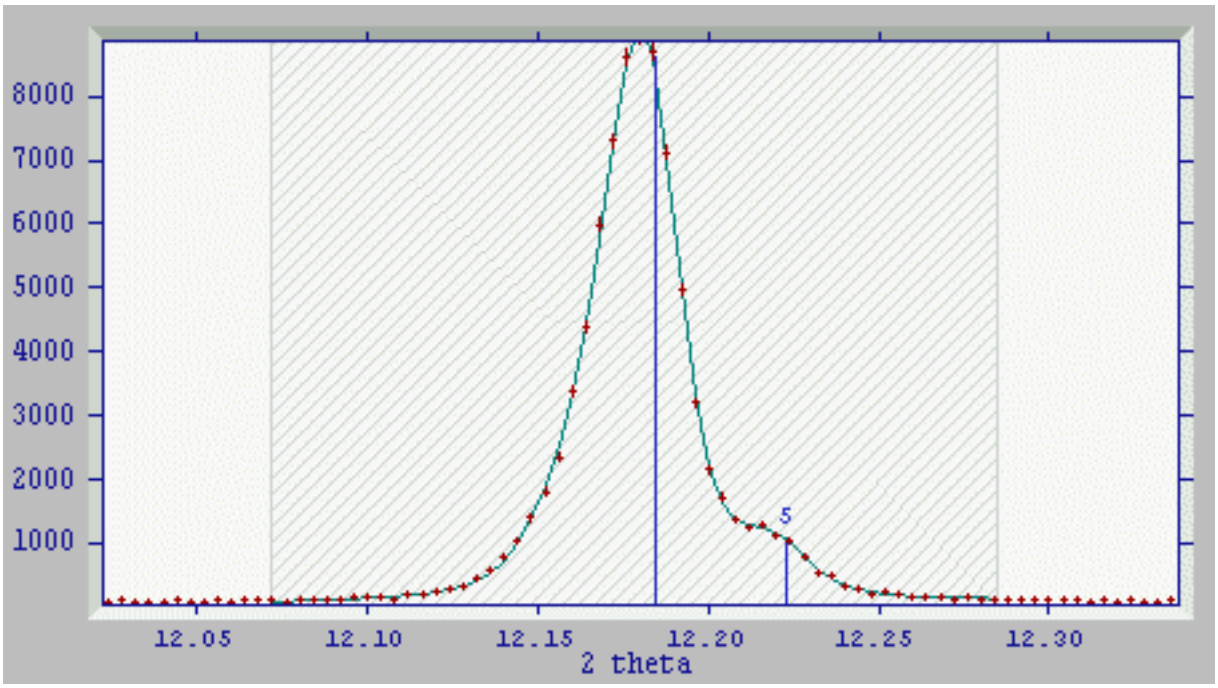
- Do not worry if you do not have the exact same position, however it should be very close to this value.
- Fit the next two peaks at around 9.5 and 10.3° 2q in the same way.
- Zoom in on the doublet at 12.17° 2q. It is clear from the shape of the peak that there are two contributing reflections here.
- Sweep out an area covering the two peaks using the right mouse button.



- Now, you need to give two initial estimates for the peak position. This is easily done by moving the cursor close to the top of the first peak, and pressing **1** on the keyboard to insert the first estimate, then moving to the top of the second peak and pressing **2** to insert the second estimate.



- Then, with the cursor inside the hatched area, press **Return** or **Enter** as before to fit the two peaks. Note that the peak positions are refined from your initial estimates, in order to give the best fit to the data.



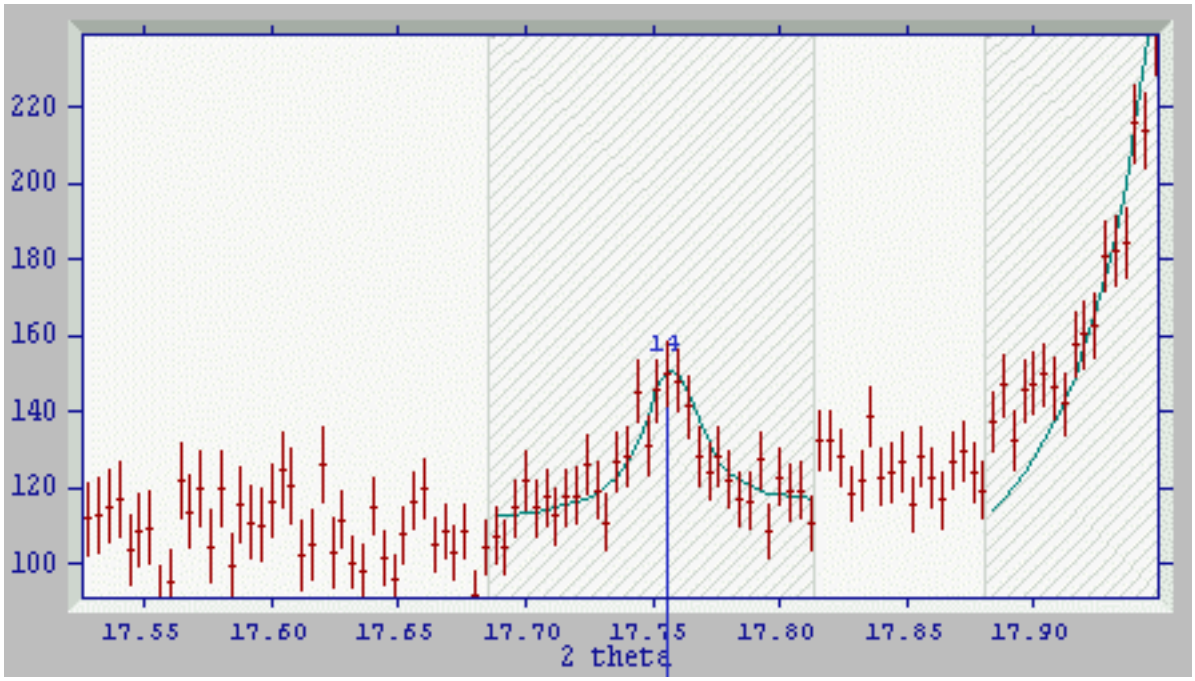
- Now, you simply repeat this until we have 20 accurate peak positions. Listed below are 2q ranges and the number of peaks contained in them as a guide.

Region / ° 2q	Peaks in region	Cumulative peaks
below 13.5	5	5
13.5 to 14.6	3	8
14.6 to 16.4	4	12
16.4 to 17.5	1	13
17.5 to 18.5	2	15
18.5 to 20	4	19
20 to 20.9	3	22
20.9 to 21.3	2	24

- Here are the first 24 peak positions as returned by DASH, viewed by selecting **Peak Positions** from the **View** menu:

6.9822	9.4942	10.3453	12.1847	12.2228
13.6925	13.7905	14.0003	15.2696	15.6883
15.7753	15.9581	16.8146	17.7552	18.0107
19.0501	19.1452	19.3479	19.7249	20.5468
20.6314	20.7735	21.0639	21.1688	

- The only peak you might have struggled to see was the one at ~17.75° 2q, as it is very weak.



15.1.6 Stage 4. Indexing

- Having selected 20 or so peaks we now want to index the pattern. There are an number of options at this point, you can choose to index the pattern using the installed version of DICVOL, use an external program (McMaille or DICVOL04) or enter known unit cell parameters. **Index pattern** is already selected so click **Next >** to index using the internal version of DICVOL.
- Ensure that all crystal systems except Triclinic are selected.
- Select **Run >** to run the DICVOL indexing program (See the lists of available software for powder indexing at *http://www.ccp14.ac.uk/solution/indexing/*).

For other Indexing Programs you can easily get the peak positions out of DASH and into a file by:

- Selecting **Peak Positions** from the **View** menu and then clicking on the word **Position** at the top of the column containing the peak positions. This selects the entire column.
- Use **Ctrl+C** to copy the entire column to the clipboard.
- Inside an appropriate editor, such as Notepad or Wordpad, use **Ctrl+V** to paste the column into a file.
- Save the line positions into a file with the correct format for your favourite autoindexing program.

Your indexing program should return a monoclinic unit cell of volume ~576 $Å^3$.

A typical run of DICVOL, if the selected peaks were very close to those given in the previous stage,

returns a monoclinic cell, with:

a = 9.9388 Å, b = 8.49954 Å, c = 7.31875 Å, b = 111.19°, V = 576.453 $Å^3$

Figures of merit: M(24) = 131, F(24) = 446

With figures of merit as good as these, there is little doubt that the cell has been correctly indexed. It is possible to change this cell into one with a conventional setting, but for the moment, we will proceed with the cell as it is returned by DICVOL.

15.1.7 Stage 5. Stop and Think

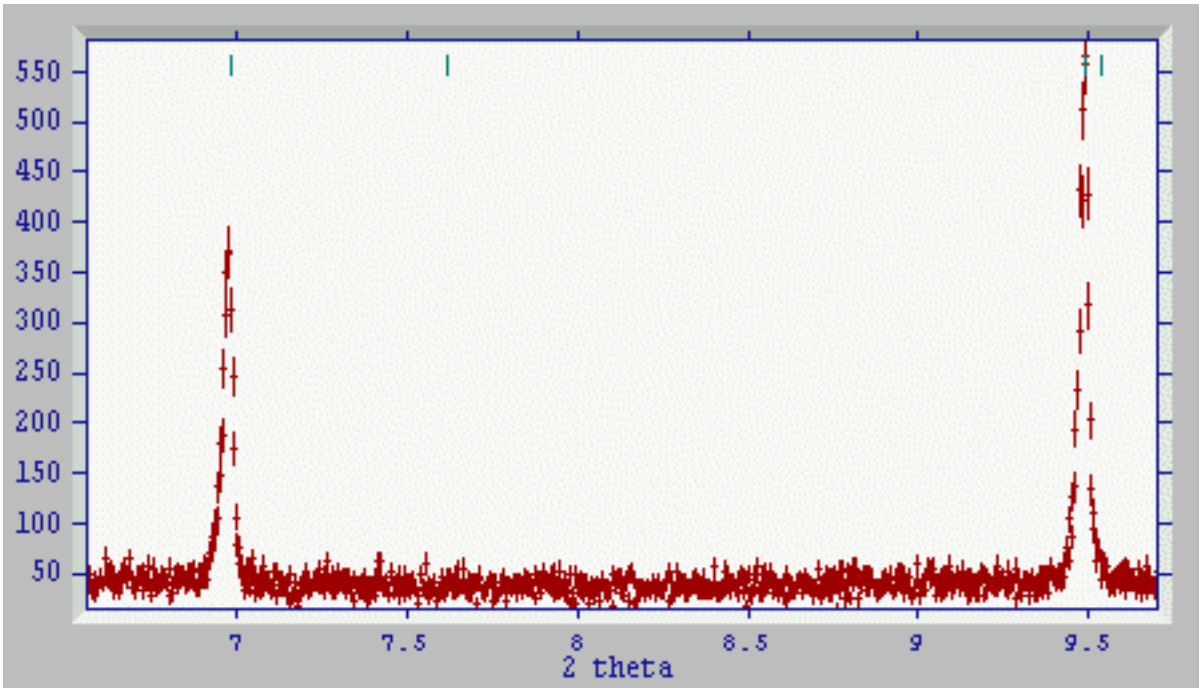
Does the cell make sense? There is a very approximate method of estimating molecular volume using 15 $Å^3$ per C, N, O atom, 25 $Å^3$ for Cl, S, and 5 $Å^3$ per hydrogen atom. So for this molecule $C_7H_4N_3O_2S_2Cl$ we estimate the molecular volume to be 275 $Å^3$, so 2 molecules per cell would need a volume approximately 550 $Å^3$. The DICVOL cell volume of 576 $Å^3$ suggests that we have two molecules per cell, and given that the cell is monoclinic, a likely space group is $P2_1$, since $Z = 2$ for this space group.

15.1.8 Stage 6. Checking the Cell and Determining the Space Group

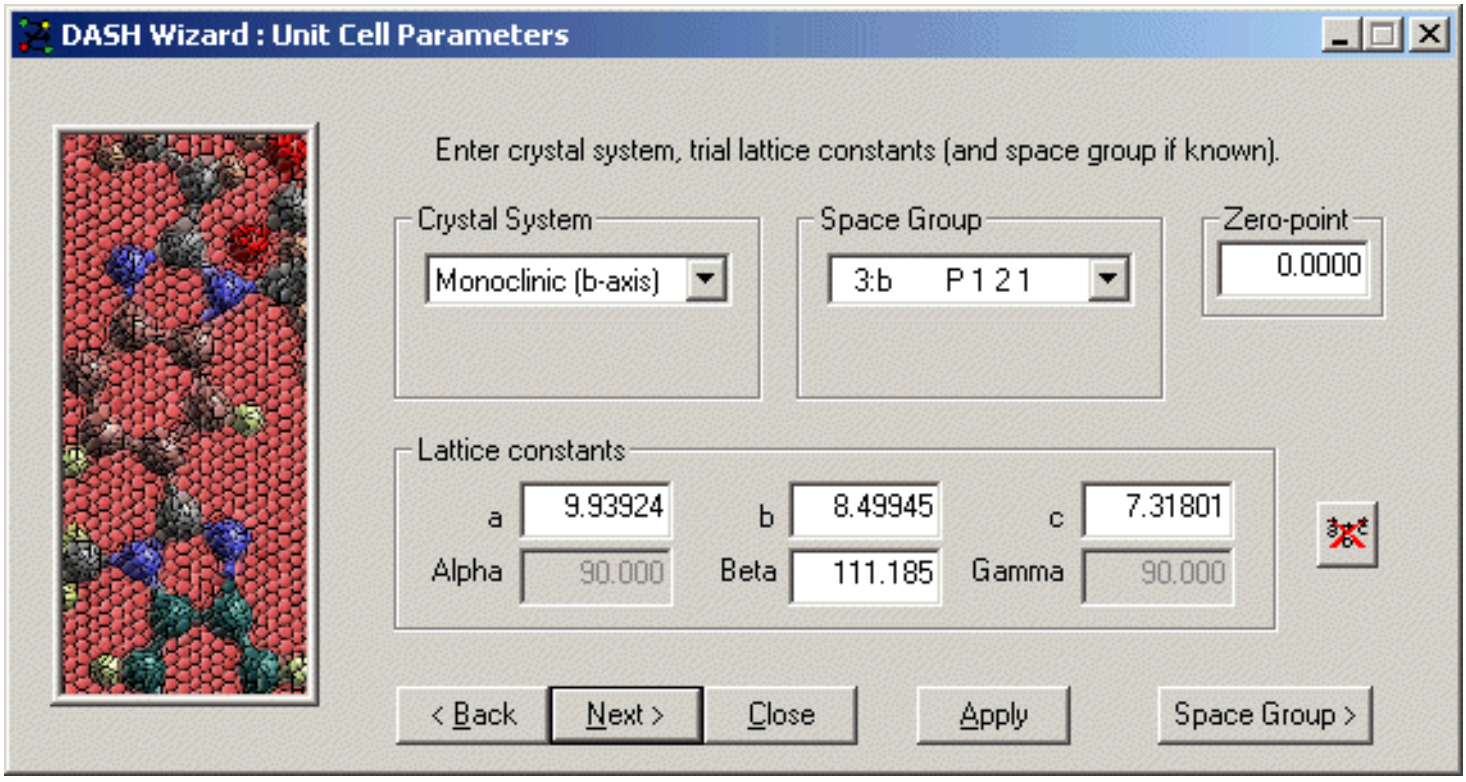
- Select the top solution from the *Results from DICVOL* window and import this into DASH by selecting the button next to it in the *Import* column. If only one solution is obtained it will be imported automatically into DASH.
- Click **Next >**.

You will see the data displayed as before, but this time, there are a series of tick marks at the top of the plot to indicate where the Bragg reflections corresponding to the input cell occur.

- The first thing to do is to ensure that in general, the tick marks correspond to peaks within the pattern.
- Any unaccounted for diffraction peaks are a warning that the determined unit cell might not be correct, or that there is an impurity phase present.
- A quick glance at the *Tutorial_1.xye* pattern shows no unaccounted for peaks, but a few excess tick marks. For example:

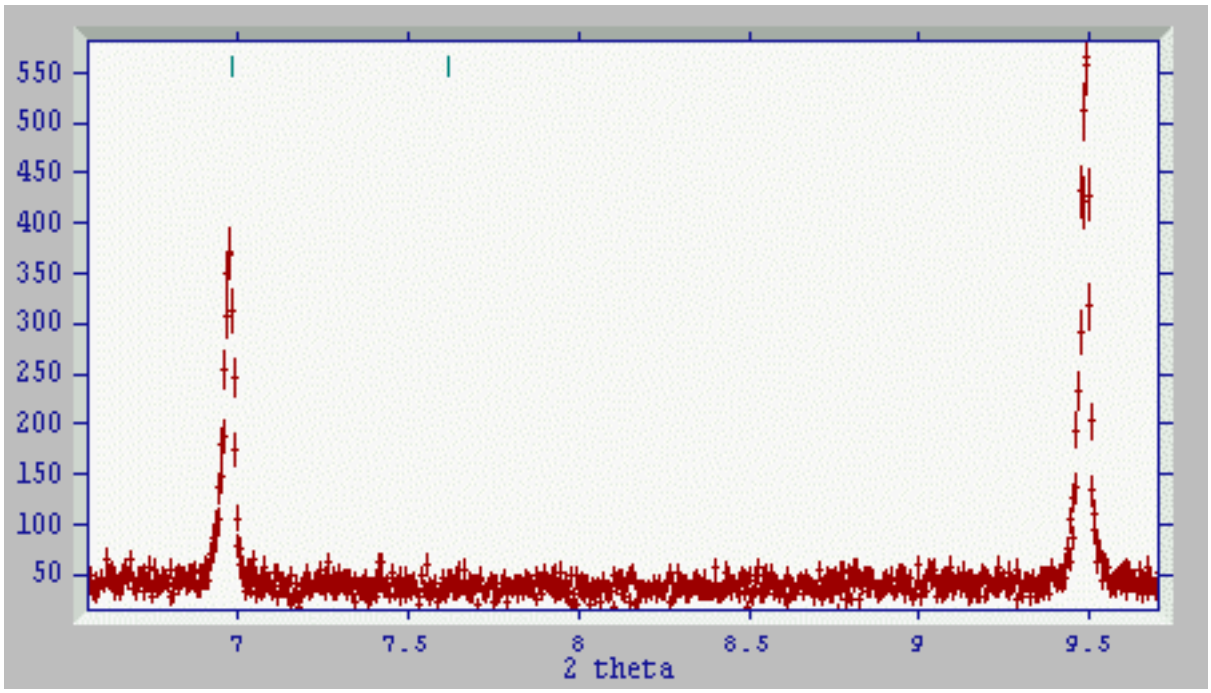


- The tick at just over 7.5° 2q does not appear to correspond to anything other than background intensity, which means that it probably corresponds to a systematic absence for the true space group of the crystal.
- The tick at just over 9.5° 2q may be another absence, although there is just a hint of a shoulder present on the stronger peak.
- We already guessed that a likely space group is $P2_1$, so let us see if increasing the symmetry from $P2$ to $P2_1$ eliminates likely absences whilst leaving no unaccounted for peaks.
- In the *Unit Cell Parameters* window select $P2_1$ from the *Space Group* pull down menu:



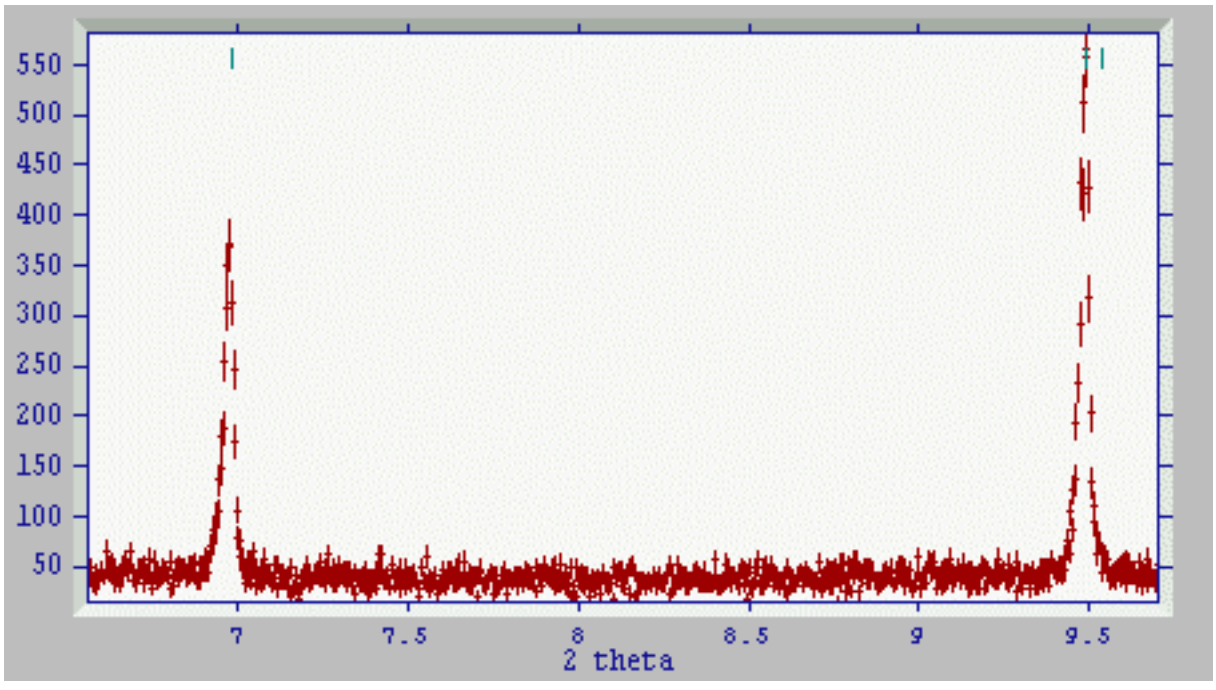
- Using the down arrow cursor key, move down the list, and watch the tick marks update to show the reflection positions corresponding to the currently selected space group. Alternatively, you can use the mouse to scroll down and select individual space groups.
- It's pretty obvious that choices such as *P* 1 *c* 1 eliminate major peaks and clearly cannot be correct.

Tick-marks with space group *P* 1 *c* 1:



- Alternatively, *P* 1 2_1 1 eliminates the tick at 7.5° 2q whilst leaving one at just over 9.5.

Tick-marks with space group *P* 1 2_1 1:



- Examining the rest of the pattern, the correspondence between tick marks and peaks is excellent and we can conclude that the peak at just over 9.5° 2q is a very weak diffraction feature of a crystal whose space group is $P2_1$, b axis unique.
- Click **Apply** in order to select the space group $P2_1$.
- Click **Next >.**

15.1.9 Stage 7. Extracting Intensities

Initially this is much like the indexing phase. We are aiming to model the entire diffraction pattern and so we need to be able to fit peaks. We are confident that we have a reasonably accurate cell and the correct space group. The criteria for peak fitting are slightly different from the ones used in indexing and we need to:

- Fit a number of, preferably, isolated reflections.
- Sample peaks across the pattern in order to parameterise the peak shape across the pattern.
- Ensure that any peak asymmetry is modelled at the start of the pattern.

From the *Pawley Refinement Step 1: Peak Picking* window select **Clear Peaks**. Some suitable peaks for this pattern are given below. Fit them by sweeping out areas over the peaks with the right mouse button as before in the order they are given.

Peak	Approx. Location	Note
1	6.97	
2	9.49	
3	14.0	[1]Option to Pawley refine
4	16.8	
5	20.78	
6	22.75	[2]L/parameter refine starts
7	27.8	
8	31.85	[3]Pawley window appears

1) After three peaks have been fitted, you are given the chance to go to profile refinement directly by pressing the following icon:

or choosing **Pawley Refinement** from the **Mode** menu. Ignore this option for the moment.

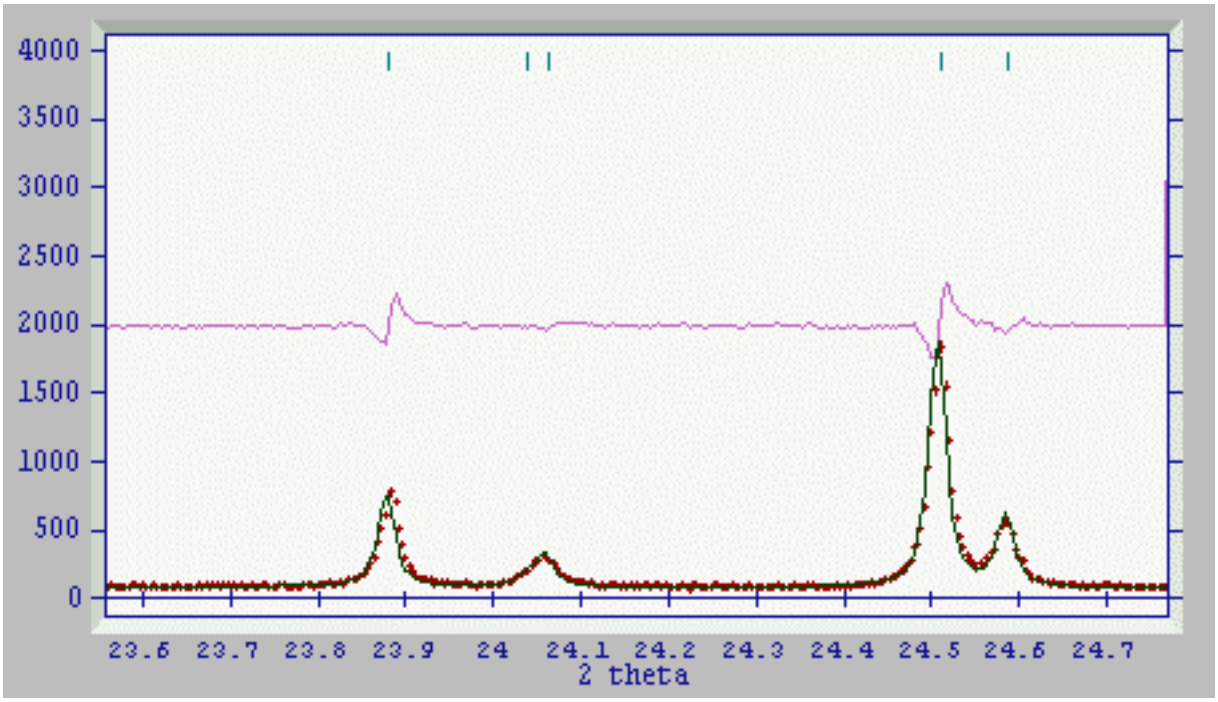
2) After six peaks have been fitted, DASH has sufficient information to allow a lattice parameter (4 parameters + zero point) refinement. The results of the refinement can be seen by selecting **Peak Positions** from **View** menu. This improves the lattice constants in the majority of cases and greatly improves the starting position for the Pawley refinement.

3) After eight peaks have been fitted, DASH has determined that the peak shape has been sufficiently well defined to allow a full Pawley refinement to be performed. The *Pawley Refinement Step 2* window will pop up automatically.

- In the initial Pawley refinement, only the terms describing the background and the terms corresponding to individual reflection intensities are refined, using the previously refined unit cell and zero-point.
- Select **Refine**; 3 cycles of least squares are performed.
- This should return figures similar to the ones below (or better).

204 reflections 9751 points Rwp = 22.25 R(exp) = 9.38 c^2 = 5.6

- Click **Accept** to accept the results of this refinement, the fit is then displayed.
- Now click in the main window and press **Home** to see how well the data are fitted. The (obs minus calc) plot is shown in pink and emphasises any misfit in the data. If you look closely at the data, you are likely to see something like this:

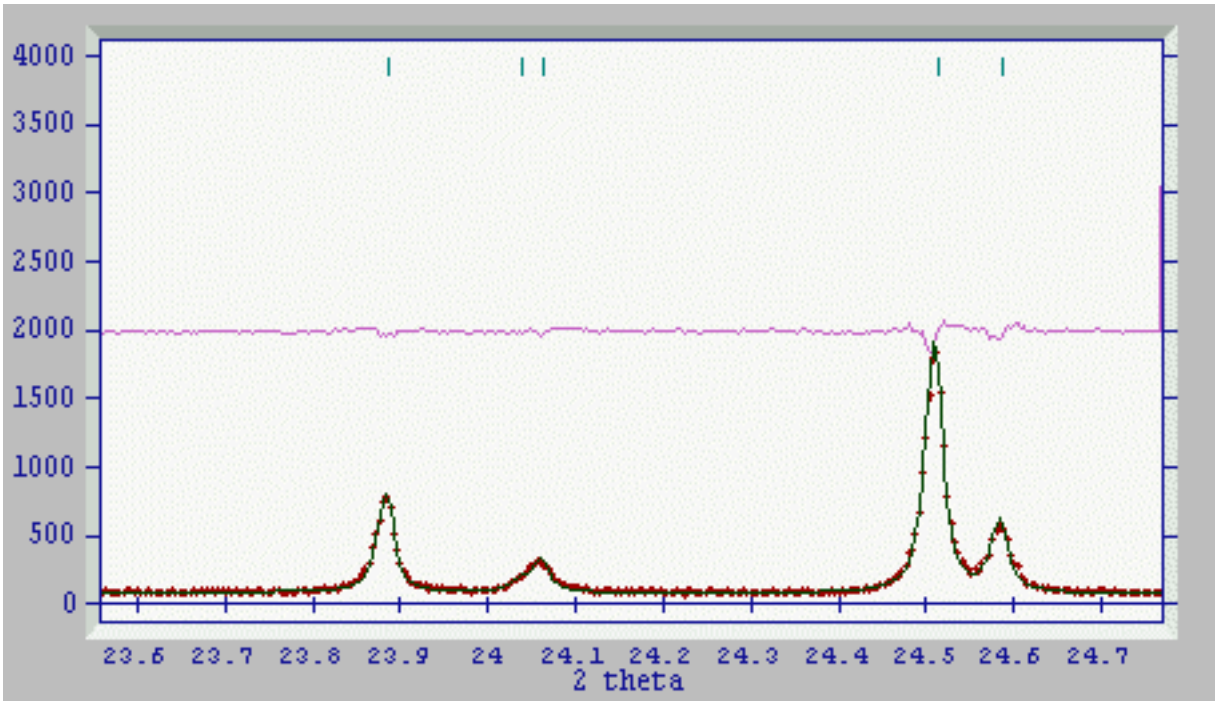


The fit is very good, but the tell-tale sinusoidal misfit indicates that the unit cell and zero point are in need of some further refinement.

- Going back to the Pawley window, note that the program has anticipated this and has flagged the unit cell and zero-point for refinement.
- Select **Refine** to perform a Pawley refinement for 5 cycles, in which the background, intensities, unit cell and zero point are all refined. The fit should improve to, for example:

204 reflections 9751 points Rwp = 16.2 R(exp) = 9.38 $c^2 = 3.0$

- The figures of merit have improved, select **Accept** to see the improvement in the fit.

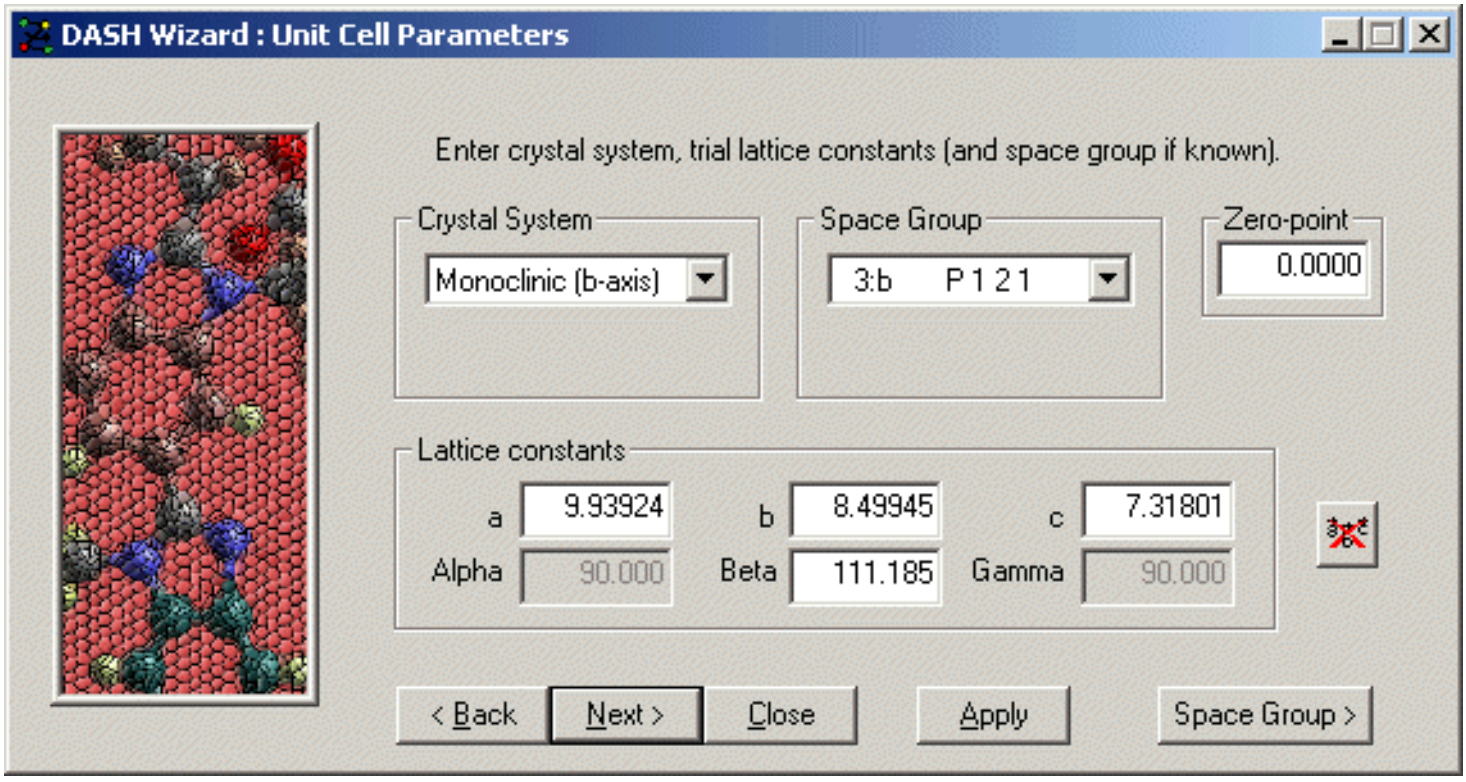


- Examine the whole profile. If you have achieved a c^2 of around 3, the fit to the data will be excellent. Click **Save as** to save the refinement results to disk as a DASH Pawley-Fit File (`.sdi`) called *Tutorial_1.sdi*.

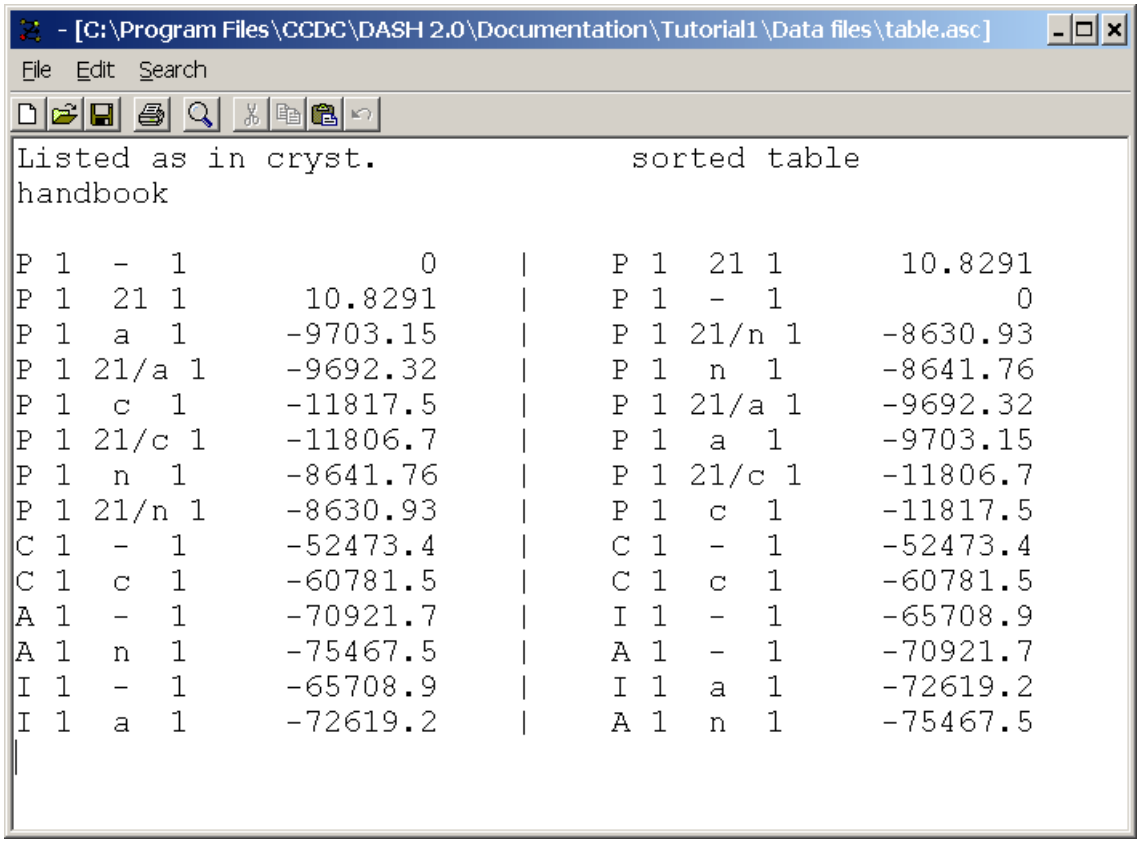
15.1.10 Stage 8. Revisiting Space Group Determination

Now that Pawley fitting and refinement has been introduced it is an appropriate point to try an alternative way of determining the space group.

- Select **< Back** in the *Pawley Refinement Step 2* window to return to the *Unit Cell Parameters* window. This time, select **Space Group>**.



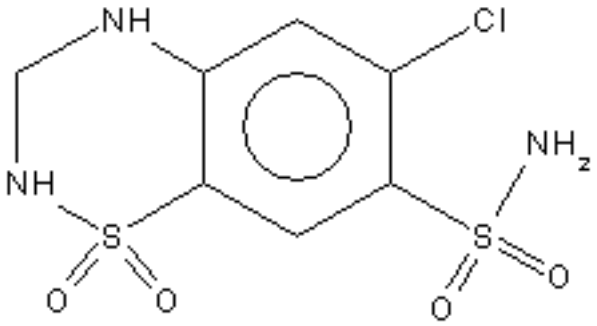
- In order to furnish the space group determination program with a required set of reflections and intensities, a Pawley fit to the profile has to be obtained in the most general space group of the crystal system under study. On pressing **Space Group>** DASH automatically selects the correct space group.
- Select **Clear Peaks** from the *Pawley Refinement Step 1: Peak Picking* window and proceed through the Pawley fitting process as before. Once a good Pawley fit to the data has been achieved, press **Run>** to launch the space group determination program.
- The console window of *Extinction Symbol* will appear and once the calculations have finished, press **Enter** on the keyboard to view the results. The space group P21 should be the most probable space group found for the data and hence will be listed first with the highest probability in the right hand column of the results table:



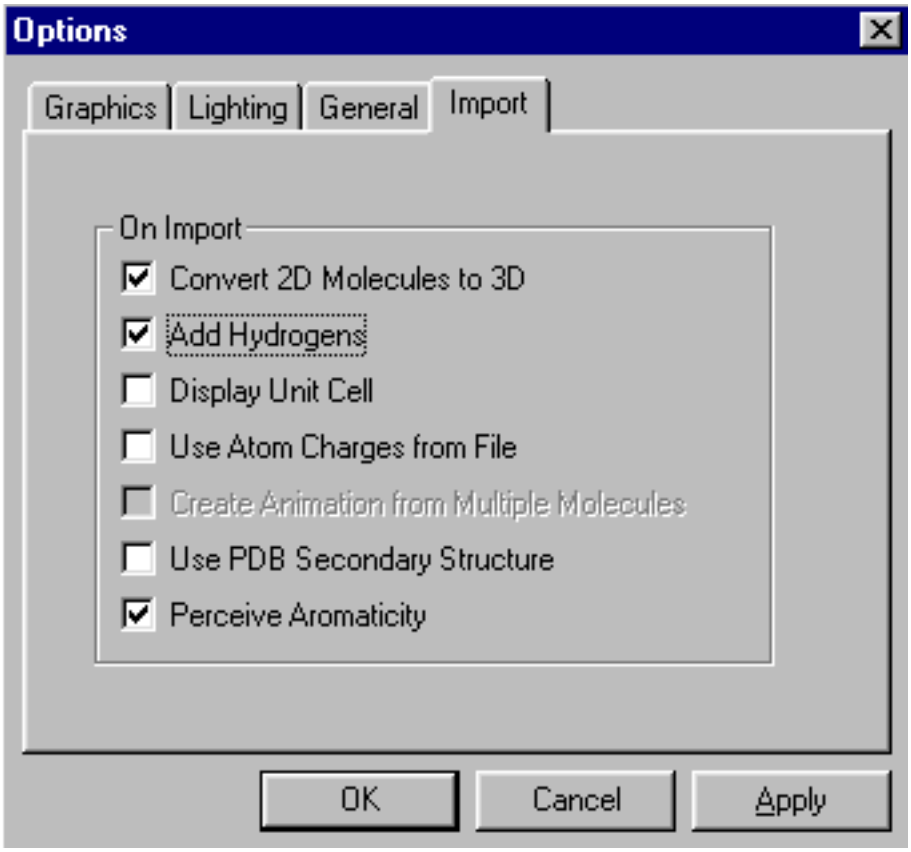
- Close the window showing the results and press **<Back** in the *Pawley Refinement Step 2* window. A dialogue box will pop-up asking whether the files generated during space group determination should be removed: select **Yes**. Now from the *Space Group* pull down menu select the space group determined to be the most probable (P21) and press **Apply**. Check the correspondence between the tick-marks and the peak positions as before.
- The correct space group has been chosen and hence a Pawley refinement should be performed in this space group. This step has already been performed previously and the `.sdi` file saved. Hence the structure solution process can begin.
- You can exit DASH at this point, if you wish, by selecting **Exit** from the **File** menu.

15.1.11 Stage 9. Molecule Construction

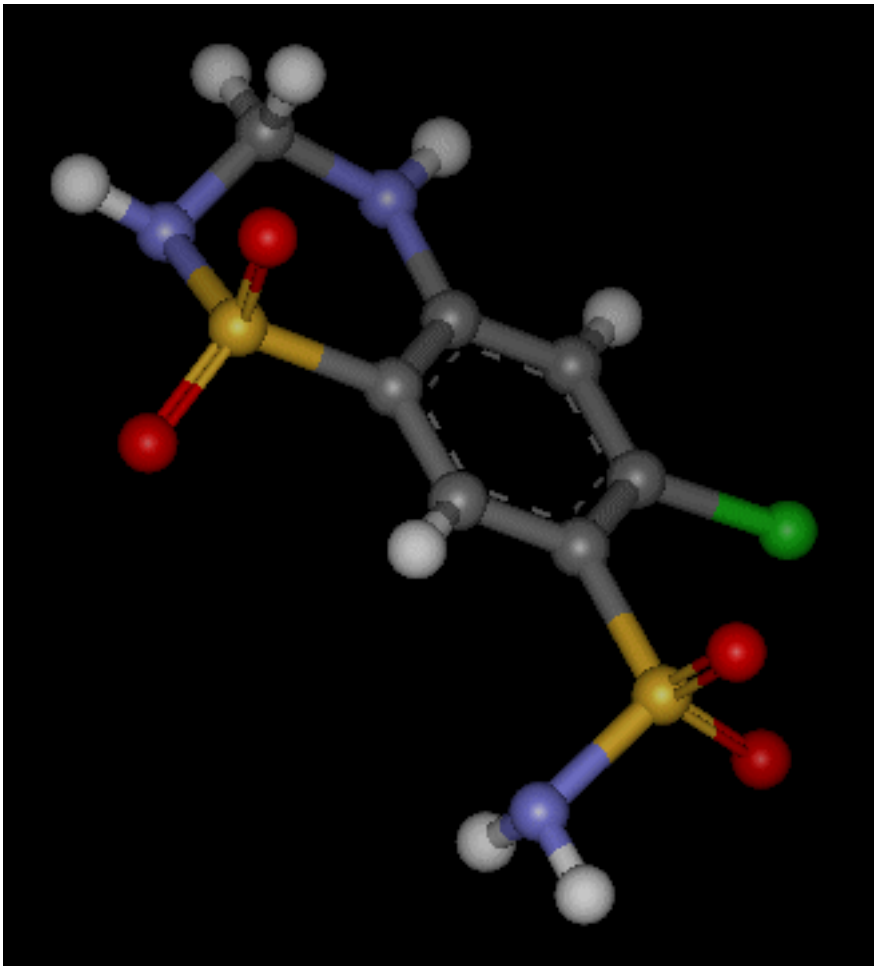
The crystal structure of the molecule that we are trying to solve is given below:



- You need to construct a 3D molecular description of the above molecule using your favourite modelling software and save it in pdb, mol or mol2 format.
- If you do not have a modelling package to hand, there is a model file named *Tutorial_1.mol2* provided with the tutorial, taking the co-ordinates from the Cambridge Structural Database reference code HCSBTZ. If you are using this model rather than creating your own you can now skip to Stage 10.
- For the purposes of the tutorial, we'll assume that the molecule was sketched (as indeed it was) using the freely available ISIS/Draw sketching package.
- Furthermore, we will assume the 2D to 3D conversion will be performed using the widely available WebLabViewer.
- Once the molecule is sketched within ISIS/Draw, select the whole molecule and copy it to the clipboard using **Ctrl+C**.
- Within WebLabViewer, ensure that the following **Import** options (accessed from the **View** menu) are enabled.



- Paste the 2D model into WebLabViewer using **Ctrl+V**.
- After pasting the molecule into WebLabViewer, the 2D chemical sketch is converted into a 3D molecular model (in ball & stick display mode):



- Save the molecule coordinates in mol format as *Tutorial_1.mol*.

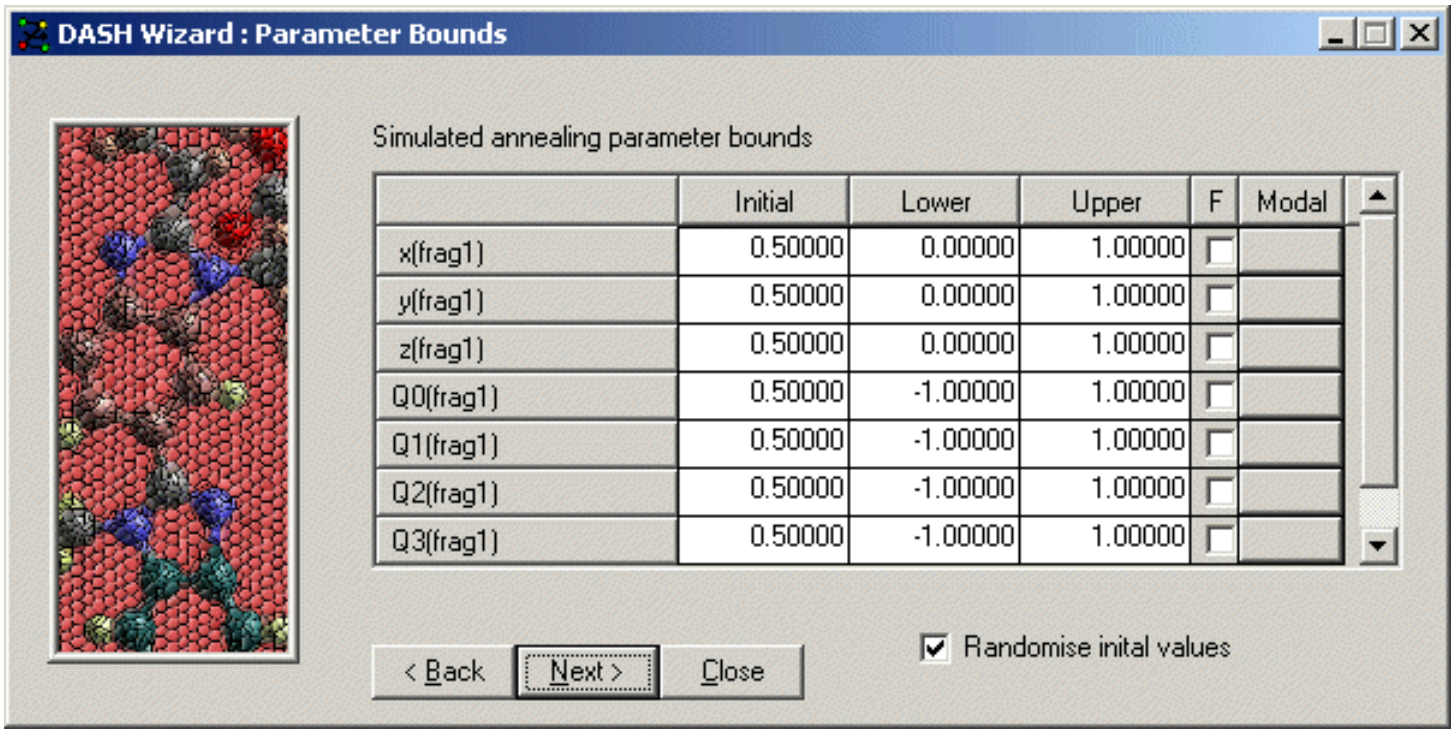
15.1.12 Stage 10. Setting up the Structure Solution Run

- Start DASH as before and select **Simulated annealing structure solution** from the Wizard. Click **Next >.**
- Browse for the DASH project file that you saved at the end of Stage 7 and load the file that you saved, e.g. *Tutorial_1.sdi*.
- Click on the  icon and read in the *Tutorial_1.mol* file, or the *Tutorial_1.mol2* file.
- DASH will generate the internal format (Z-matrix) that it uses to describe the molecular conformation.
- DASH analyses the molecule and automatically selects rotatable torsions. In this case, the bond connecting the benzene ring to the SO_2NH_2 group is the only rotatable torsion in the molecule.
- Read in the newly created Z-matrix by clicking on the  icon in the *Molecular Z-Matrices* window and selecting the file *Tutorial_1.zmatrix*.

- Note that DASH has determined that there are 7 independent degrees of freedom to be determined if the crystal structure is to be solved i.e. 3 positional coordinates for the centre of mass of the molecule, 3 parameters describing the orientation of the molecule within the unit cell, and 1 internal torsion angle describing the molecular conformation.
- DASH now has the information it needs concerning the molecule, so click **Next >**.

The following menu allows you to fix or bound parameters. In this particular example, we are allowed to fix the y coordinate of the centre of mass of the molecule at any position, as $P2_1$ is a polar space group.

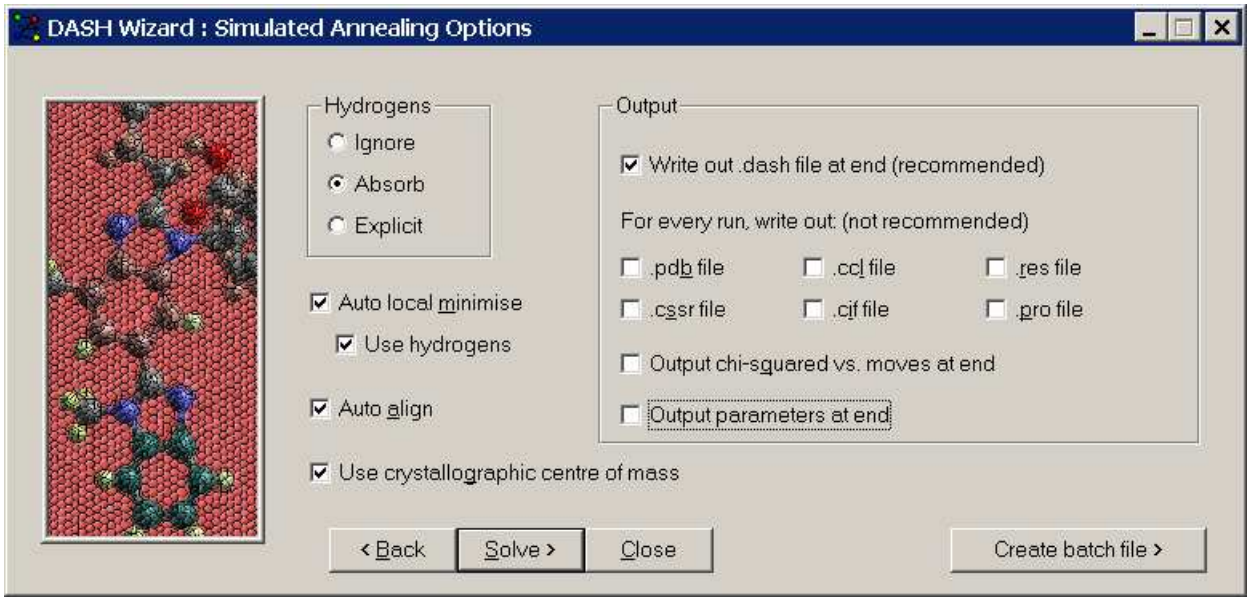
- Do this by clicking on **F** (short for fix) in the line corresponding to the y coordinate of the molecular fragment (*y(frag1)*):



- Click **Next >**.

The resulting *Simulated Annealing Protocol* window that appears need not concern us here. In most cases, the default values will suffice. See the DASH User Guide for more details. Click **Nex t >**.

The next window asks you to choose some options for the Simulated Annealing procedure. It is useful at this stage to go over some of the details.



- **Hydrogens:** as the scattering power of hydrogens is low, hydrogens are ignored by default to speed up the calculations. The **Absorb** option absorbs the electrons from the hydrogen in their riding atoms. For single crystal data, the hydrogen atoms can be taken into account explicitly during the SA.
  Note:
  By default hydrogens are always included during the local minimisation at the end of each simulated annealing run.
  During Rietveld refinement, hydrogen atoms are always included.
- **Use crystallographic centre of mass:** when selected each atom is assigned a weight of Z^{-2} when the molecular centre of rotation is calculated, where Z is its number of electrons. Otherwise, no weights are applied.
- **Auto local minimise:** when selected, the c^2 of each final solution is minimised using a simplex algorithm before the solution is written out. If **Use hydrogens** under **Auto local minimise** is selected, hydrogens are included in the local minimisations of the final solutions.
- **Auto align:** when selected the molecules of the final solution are aligned before the solution is written out. This only applies when more than one run has been selected.
- **Output .pdb**: when selected the crystal structure of the final solution is written out in pdb format.
- **Output .cssr:** when selected the crystal structure of the final solution is written out in cssr format.
- **Output .ccl:** when selected the crystal structure of the final solution is written out in ccl format.
- **Output .pro:** when selected a file with the extension. `.pro` is written out which contains 2q, the observed profile, the calculated profile for the best solution and the original esds. The file is written out in ASCII format and can be imported into a spreadsheet package such as Excel.
- **Output .cif**: when selected the crystal structure of the final solution is written out in cif format.

- **Output .res**: when selected the crystal structure of the final solution is written out in res format.
- **Output chi-squared vs. moves:** when selected a graph of the profile c^2 versus moves is written out to a file in ASCII format with the extension `.chi`, at the end of the simulated annealing, This can be imported into a spreadsheet package such as Excel.

Note that when more than 1 simulated annealing run is requested, the above options pertain to every run. This can generate quite a number of files. Each option (except **Use hydrogens**) can be switched on and off while the simulated annealing is running.

- Click **Solve >**.
- The simulated annealing run now starts.

15.1.13 Stage 11. Monitoring Structure Solution Progress

Full details of all the output from the structure solution run are given in the DASH User Guide. For this tutorial, you need only watch:

- The Profile c^2.
- The (obs - calc) plot i.e. the difference plot, shown by default in pink.

The profile c^2 is on the same scale as the Pawley fit profile c^2 that you obtained when fitting the data in Stage 7. So if the current profile c^2 is close to the value of the Pawley profile c^2, you've probably solved the structure.

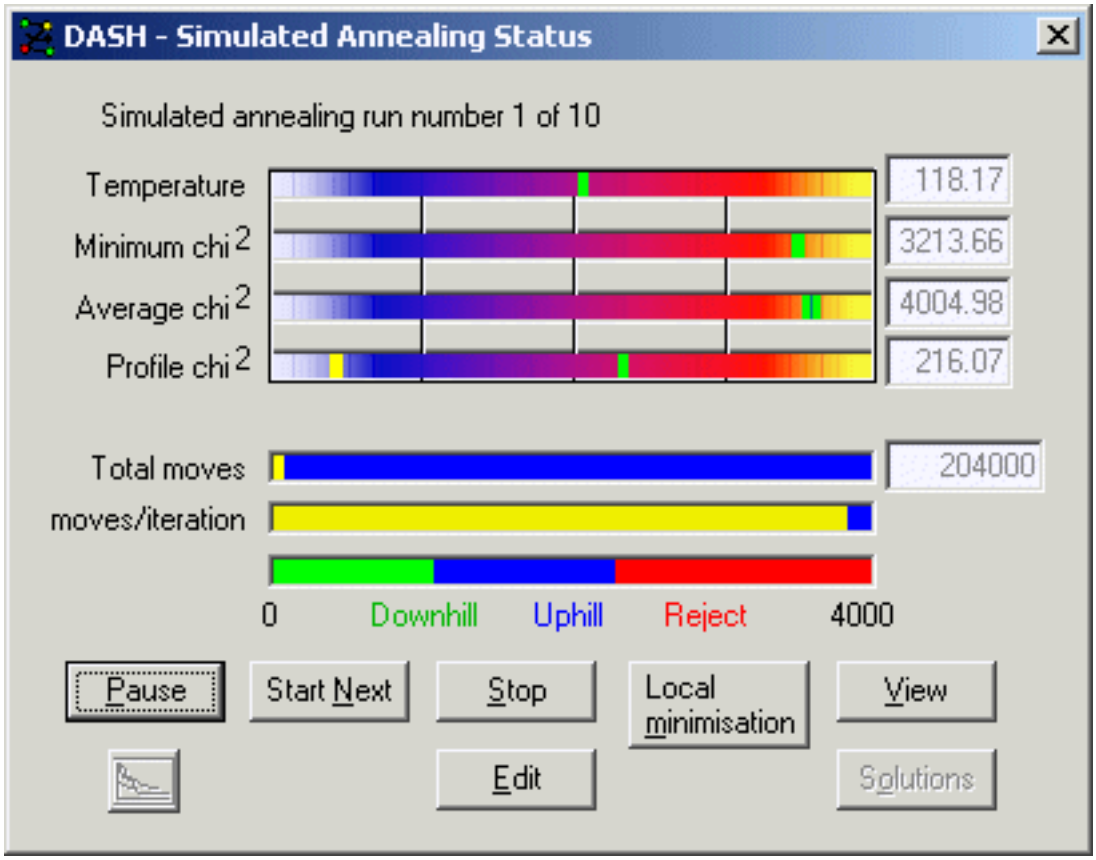
DASH runs the SA process until the user intervenes by pressing one of the following buttons on the SA output panel:

- **Pause** - Pauses the SA run until you hit **OK**. This can be useful to free up the processor temporarily, as DASH is computationally intensive.
- **Start next** - When in a multi-run, the **Start next** button terminates the current run and starts the next one.
- **Stop** - Stops the simulated annealing run immediately and returns you to the first *Simulated Annealing Protocol* window.
- Click **Edit** to end a simulated annealing run and change parameters.
- **Local minimisation** - Invokes a simplex optimisation that takes the structure to the deepest minimum in the vicinity of the current best structure.
- Click **View** to visualise the crystal structure of the best solution obtained so far for the current run.

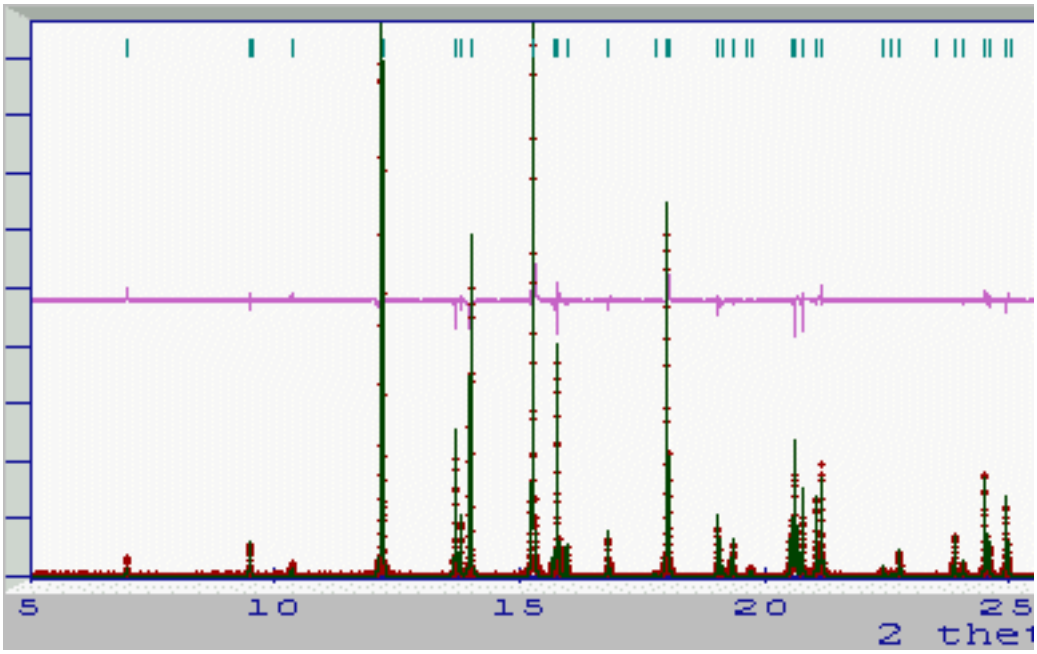
- Click **Solutions** to analyse the solutions found so far.

On a modestly specified PC (e.g. Pentium III 300 MHz) the structure solution process should take less than 30 seconds to reach a profile c^2 of around 12, by which point the structure is solved to a high degree of accuracy (this is an ideal value the actual number you get may differ from this).

The profile c^2 is 12.05, less than four times that of the Pawley profile c^2, i.e. solved:



Examine the difference plot:



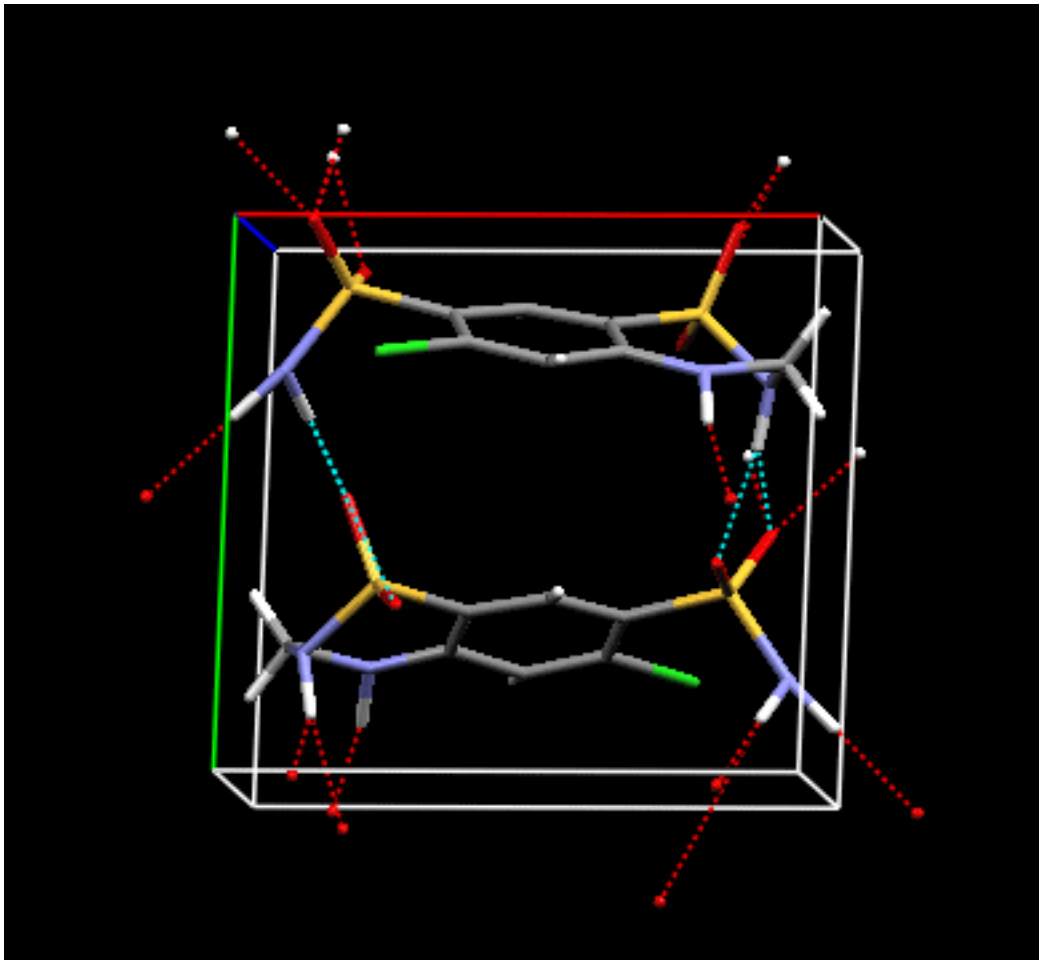
- The fit is excellent, even at high angle.
- Remember also that we have effectively only refined a scale factor to get to this point and the structure is clearly solved.
- Click **View** to see the solution in the 3D-visualiser.

15.1.14 Stage 12. Examining the Output Structure

DASH can output 5 coordinate file formats describing the final answer output from simulated annealing (see the *Configuration...* window). Here, we assume that the project filename was *Tutorial_1.sdi*.

- *Tutorial_1.pdb*: protein data bank format file containing a Cartesian coordinate description of the SA solution.
- *Tutorial_1.cssr*: Cambridge Structure Search and Retrieval format file containing a fractional coordinate description of the SA solution.
- *Tutorial_1.ccl*: Cambridge Crystallographic Subroutine Library format file containing a fractional co-ordinate description of the SA solution.
- *Tutorial_1.cif*: Crystal Information File format file containing a fractional coordinate description of the SA solution.
- *Tutorial_1.res*: SHELX format file containing a fractional coordinate description of the SA solution.

The Mercury visualiser supplied with DASH has options to display Packing and H-bonds. Using these options your answer should look like that given below. You can see that all donors and acceptors are satisfied:



Remember that the exact location of your molecule along **b** depends upon where you anchored the molecule. In the above picture, the molecule was fixed at y = 0.5. The solution obtained is in excellent agreement with that reported for hydrochlorothiazide at room temperature by Dupont & Dideberg (1972).

15.1.15 Stage 13. Rietveld Refinement

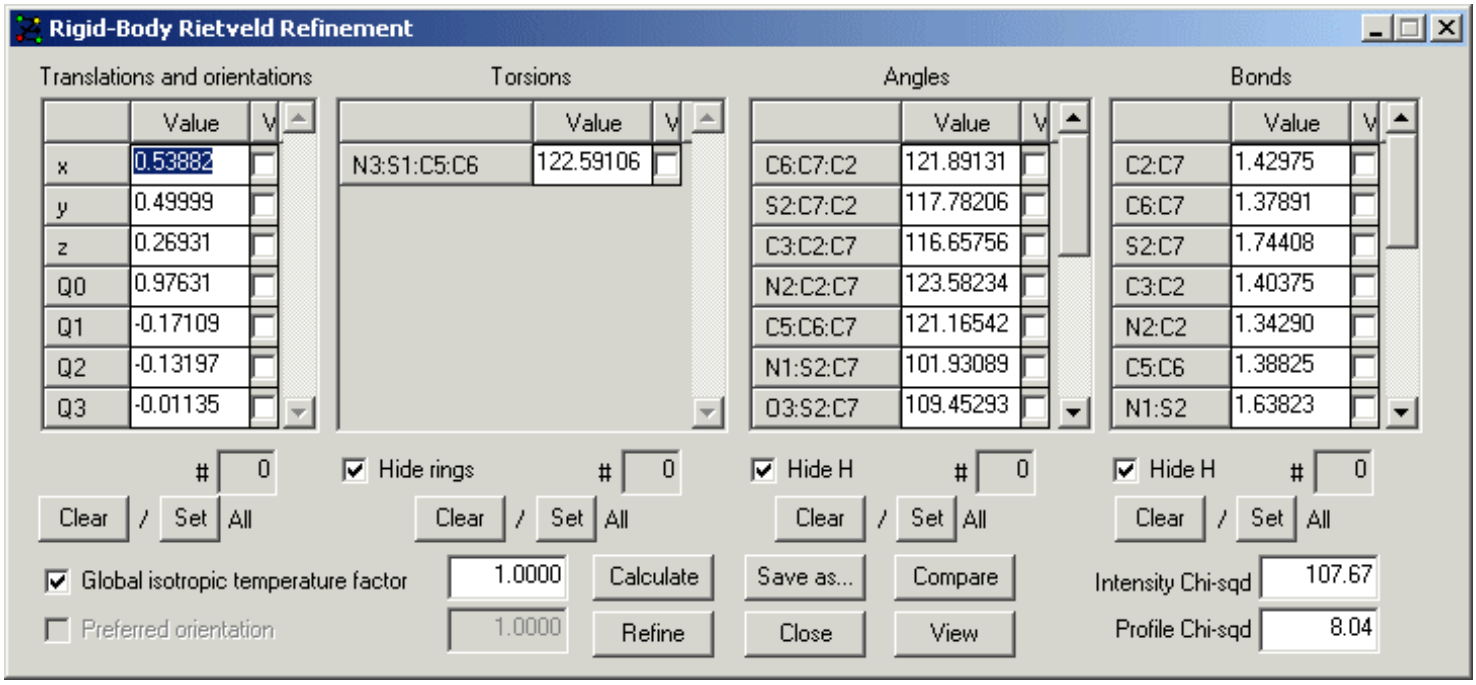
- There are several options for Rietveld refinement in DASH including interfaces to external refinement packages and a built-in module for refinement. For the purposes of this example, to refine the structure in a meaningful manner we will use the built-in rigid-body Rietveld refinement module (see Section 11.1 of the DASH User Guide). This implementation uses the extracted intensities with their correlation matrix from the Pawley refinement stage, and is thus limited to the resolution chosen in 2q in Stage 1. Up to now the default setting in DASH has been used which truncated the data to 2q of 37.65, corresponding to a resolution of 1.75Å.
- Examination of the Pawley refinement carried out in Stage 7 will show the actual number of

extracted intensities e.g. 135. The Pawley refinement result values may be examined using the **View** menu, and then selecting **Pawley / SA**. The low resolution of the data means that we cannot expect to refine coordinates of this molecule in an unconstrained manner and obtain physically reasonable values. The information is just not there!

- However, it is meaningful to fit the molecule as a sequence of rigid-body fragments, which is the meaning of the Z-matrix description. The Z-matrix consists of *instructions* for constructing the molecule atom-by-atom, each atom in this case being added according to the values given for a bond-length to a preceding atom, a bond-angle to two preceding atoms, and finally a torsion angle to three preceding atoms.
- In Stage 10 you ran a program which converted the Cartesian model of Stage 9 into a Z-matrix. The program further identified, by examination of the chemical atom types, that only one torsion angle should be treated as variable, namely *N3:S1:C5:C6*. The SA run explored the search space with all other parameters of the Z-matrix fixed, which we have seen this gives a chemically sensible result in our solution. The Rietveld refinement allows variation of any of the Z-matrix parameters, for the chosen solution.

- The first stage of the Rietveld refinement is to keep all parameters fixed except the global isotropic temperature factor scale. This allows the initial guess of the Biso values for each atom in the Z-matrix to be adjusted by a global scale factor; the default values set by DASH are Biso = 3.0 for all non-hydrogens and 6.0 for hydrogens. When the scale factor, K, is refined then the Biso for each atom is simply K.Biso as input in the Z-matrix file.
- In single crystal X-ray refinement it is usual to set the value for Biso for hydrogens to about 1.25 times the parent heavy atom. In the case of powder data, for this size of molecule, there will be little observable effect if we set all H-atoms to have the same value. The starting Biso hydrogen value of 6.0 may be considered rather too large and could be adjusted to say 4.0. However the value of Biso for non-hydrogen atoms is typical for this size of molecule at room-temperature.

- Choose the first solution in the *Analyse Solutions* dialogue box, click on the **Rietveld** then select *Rigid-body Rietveld refinement* and click on **Next >**, this will launch the following window:



- You will see that the check-box for **Global isotropic temperature factor** is selected, click **Refine**. The starting value of 1.000 with Chi-sqd 100.72 and Profile Chi-sqd 8.01 will change to 0.13902, 43.49 and 5.53 respectively. This very low value of the temperature factor scale corresponds to the unusually low data collection temperature of 20K. Keep this temperature factor fixed by deselecting the check box.
- Now switch on the check boxes marked *V* for the left hand column *Translations and orientations* by clicking the **Set** button below this column. This specifies refinement of the translation parameter for the molecule centre of mass, and the orientation of the molecule. In this space group P21 the position along the y-axis is arbitrary, so switch off the **y** check box, and click **Refine**. You will see the Chi-sqd values drop to 42.58 and 5.48, with slight change of temperature scale to 0.13059.
- This is perhaps as far as is reasonable to refine this structure for publication. You can now output a CIF file using the **Save as...** button, e.g. with filename *Tut1-Rietveld-Biso*, and choose output file type `.cif` from the list of file formats.
- You can check that the Rietveld refinement is stable by allowing all torsion angles to vary; click the **Set** button at the bottom of the *Torsions* column, deselect the **Global isotropic temperature factor**, and clear the *Translations and Orientations* by using the **Clear** button underneath the column. There will be very little change in the parameters with almost no reduction of the Chi-sqd values.

- As a further experiment you can also switch off refinement of the *Translations and Orientations*, and *Torsions* by using the **Clear** button underneath each column, and deselect the **Global isotropic temperature factor** box. Now allow all bond angles to vary. You see a small shift in coordinates, with a small reduction in Chi-sqd to 36.82 and 5.22. You can compare this refined structure to the starting value when you entered the Rietveld window by clicking **Compare**. The Mercury display shows that there have been very small shifts in the coordinates (use the **Zoom-in** feature). The resulting coordinates are not more significant than the previous coordinates saved as the CIF file, but they do confirm that the refinement is stable, and you are in a local minimum of the Chi-sqd surface.
- Similarly you could refine all the bond-lengths, and achieve a further small reduction in Chi-sqd, but this is cannot be interpreted as an improved set of coordinates for this solution. There is simply not enough data to justify these fine variations from the starting values of bond-lengths and bond-angles. Another way of looking at the calculation is that you could achieve the same low Chi-sqd values with a range of slightly different molecular models. At present you have no information of the estimated standard deviations of the coordinates.

Note: As an experiment, that if you attempt to refine the **Global isotropic temperature factor** together with a significant number of the torsions, angles or distances that the procedure takes a considerable time to converge (e.g. 2-3 minutes), showing that the temperature factor is highly correlated with the other parameters.

- Some insight may be obtained as to how individual parameters affect the calculated Ch-sqd by manually setting any parameter value in the menu box and clicking on **Calculate**. This simply calculates Chi-sqd at that point, e.g. changing the bond-length C2:C7 from 1.4297 to 1.4397 changes Chi-sqd by typically from 28.42 to 28.73, 4.01 to 4.03. Changing the position of a heavier atom e.g. chlorine in Cl1:C4 generally produces a larger change to Chi-sqd for a shift of 0.01 in the bond-length.

15.1.16 Using maximum resolution

If this were a real example of refinement of an unknown structure, you would try to refine against the maximum resolution data that the DASH intensity extraction can handle. This tutorial example so far was carried out at a resolution of 1.75Å, using 135 extracted intensities. The current version of DASH can use a maximum of 350 reflection intensities. In order to do this you must go back to Stage 1 and read in the data with a higher truncation value in 2q, e.g. 44.0 degrees corresponding to 1.507Å resolution. This gives a Pawley fit of about 2.35 for 204 extracted reflections. When one ruins the SA there are typically solutions with Chi-sqd about 7.04. The Rietveld refinement following the order of refinement (a) Global Temperature Factor, (b) Translations and Rotations, gives a temperature factor scale 0.211 and Chi-sqd 28.42, Profile Chi-sqd 4.01.

15.1.17 To refine from another SA solution

Close the Rietveld window with **Close**, this returns you to the main DASH window. Click on the **Mode** pull-down menu, then select **Analyse Solutions**. This returns you to the list of solutions form your last SA run. Note that if you exit completely from DASH this *Analyse Solutions* window is not recoverable.

15.1.18 References

DICVOL Program:
D. Louer & M. Louer (1972) J. Appl. Crystallogr. ***5****, 271-275.*
A. Boultif & D. Louer (1991) J. Appl. Crystallogr. ***24****, 987-993.*

Extinction Symbol Program:
Markvardsen, A.J., David, W.I.F., Johnson, J.C., Shankland, K. (2001) Acta Cryst., ***A57****, 47-54*

Model Builder:
WebLabViewerLite Version 3.20 (12/8/98) Copyright 1998 Molecular Simulations, Inc.

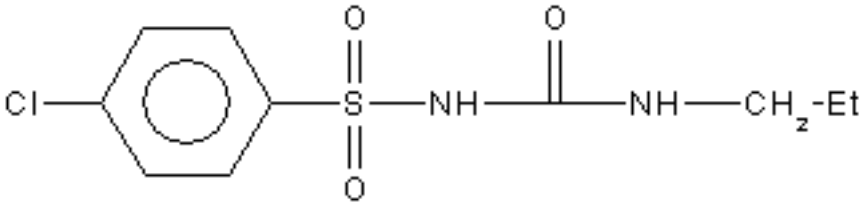
Single crystal structure (CSD Refcode HCSBTZ):
L. Dupont & O. Dideberg (1972) Acta Crystallogr. ***B28****, 2340-2347.*

15.2 Tutorial 2 - Solving a Structure from a Low Resolution Data Set

15.2.1 Introduction

The aim of this tutorial is to guide you through the structure solution of chlorpropamide and it assumes you have completed Tutorial 1. In this Tutorial you will learn how to:

- Handle structures that are more flexible than hydrochlorothiazide.
- Solve a structure from a low resolution data set.
- See one of the potential pitfalls of global optimisation i.e. local minima.



15.2.2 Data

The data set *Tutorial_2.xye* is a synchrotron X-ray diffraction data set collected on BM16 at the European Synchrotron Radiation Facility. The incident wavelength was 0.800077 Å.

15.2.3 Stage 1: Reading the data

- Open DASH and select the directory where the data resides.
- Select **View data / determine peak positions** and click **Next >**.
- Select the file *Tutorial_2.xye* using the **Browse...** button.
- Click **Next >**.
- Check that the wavelength and radiation source have been set correctly and click **Next >**.

15.2.4 Stage 2: Examining the data

Note that this data set was collected quickly at the end of a day's beamtime, and so only extends to 22° 2q. Hence the data set extends to a resolution of only ~2 Å. Truncate the data to start at 1.5° to remove the data points affected by the beam stop and then subtract the background using the default window value of 100 and click **Next >**.

15.2.5 Stage 3. Fitting the peaks to determine the exact peak positions

Select the first twenty peaks using the method described in Tutorial 1.

Here is a guide to the positions (° 2q) of the first 20 peaks:

3.4383	6.1080	6.8792	8.5344	8.9466
9.4316	9.9800	10.1033	10.2499	10.3269
10.7041	11.1635	11.3767	11.5027	12.2579
12.3053	13.3092	13.4047	13.5143	13.5696

- Click **Next >**.
- Select **Run>** to run DICVOL or use another indexing program as described in Tutorial 1.

15.2.6 Stage 4. Indexing

Your indexing program may reveal a number of possible unit cells. The unit cell with the highest figures of merit should be orthorhombic with a volume of ~1266 $Å^3$. DICVOL, for example, returns an orthorhombic cell with a = 26.66826 Å, b = 9.08435 Å, c = 5.22571 Å and volume = 1265.999 $Å^3$ with figures of merit M(20) = 107.1 and F(20) = 506.6.

Closer inspection of the other unit cells that are suggested by the indexing program will reveal that many of them are slight monoclinic distortions of the above unit cell, with almost identical volumes and lattice parameters and beta ~90°. Other suggestions generally have much lower figures of merit and can be ruled out immediately.

Considering that the orthorhombic unit cell has the best figures of merit, and that it is usually best to try the simplest option first, we will proceed to the next stage assuming an orthorhombic unit cell, with the lattice parameters given above.

15.2.7 Stage 5. Stop and Think

Does the cell make sense? In this case we estimate the molecular volume to be ~330 $Å^3$, from the formula $C_{10}H_{13}N_2O_3SCl$ and approximate volumes C, N, O = 15 $Å^3$, S, Cl = 25 $Å^3$ and H = 5 $Å^3$ in the molecule. Therefore, given the unit cell volume of ~1266 $Å^3$ we know from this very rough approximation that the cell is most likely to accommodate 4 molecules. At this point, your knowledge of space group frequencies should suggest that $P2_12_12_1$ is a strong possibility. (A list of space groups and their frequencies is given in Appendix D of the DASH User Guide.)

15.2.8 Stage 6. Checking the Cell and Determining the Space Group

The space group *P*222 will automatically have been selected. The presence of some excess tick marks indicates probable systematic absences; this means that a space group of higher symmetry might be more appropriate. Scroll through some of the possible space groups. You will see that some of the space groups can be ruled out immediately; for example, face-centred and body-centred lattices leave some peaks unaccounted for. Many of the primitive lattice space groups appear likely from the tick mark positions. In this situation, where more than one possible space group exists, it is logical to begin with the most frequently occurring space group. In this case, the most frequently occurring orthorhombic space group is $P2_12_12_1$, so select this (number 19), confirm visually that it matches the data and click **Next >**.

15.2.9 Stage 7. Extracting Intensities

Choose 7 isolated peaks from across the pattern. Fit these peaks using the method described in Tutorial 1 and then carry out the Pawley refinement. The initial 3 cycles of least squares refinement only involve the terms corresponding to the background and to the individual reflection intensities, accept these three cycles. The next 5 cycles of least squares refinement involve the terms describing background, intensities, unit cell and zero point. These refinement details will be suggested automatically by DASH.

When these cycles are complete check the difference line; this should be almost flat by this point. The final Pawley c^2 should be between 3 and 4.

Accept this Pawley fit and save it as *Tutorial_2.sdi* (Exit from DASH, if you wish, at this point in the tutorial).

15.2.10 Stage 8. Molecule Construction

Construct a 3D molecular description of the molecule using your favourite modelling software and save it in pdb, mol or mol2 format. This can be done, for example, by importing an ISIS/Draw sketch into WebLabViewer (see Tutorial 1 for further details). Save this as *Tutorial_2.pdb*, *Tutorial_2.mol* or *Tutorial_2.mol2*. (If you do not have a model building program to hand, there is a file supplied with the tutorial, *Tutorial_2.mol2*)

15.2.11 Stage 9. Setting up the Structure Solution Run

- Start DASH as before and select **Simulated annealing structure solution** from the Wizard.
- Select the *Tutorial_2.sdi* file.
- Click on the  icon and select either *Tutorial_2.pdb*, *Tutorial_2.mol*, or *Tutorial_2.mol2* (the file that you created in Stage 8); a Z-matrix file called *Tutorial_2_1.zmatrix* will be generated automatically.
- Read in the *Tutorial_2_1.zmatrix* file and click **Next >**.

Note that as $Z = 4$ for $P2_12_12_1$, it follows that $Z' = 1$ because we know from Stage 5 that the cell is most likely to accommodate 4 molecules. Therefore, only one Z-matrix is required.

At this point DASH will confirm that there are 12 independent parameters. These parameters are listed when you click on **Next >**. There are 3 parameters describing the positional co-ordinates, 4 (of which 3 independent) describing the molecular orientation within the unit cell and 6 variable torsion angles. All **F** boxes are unticked by default, indicating that all 13 parameters are allowed to vary during structure solution. Click **Next >** to proceed to the *Simulated Annealing Protocol* window. The default values can be used for this example, so click **Next >**, then **Solve >** to begin the simulated annealing.
NB: Keen chemists should resist the urge to restrict the torsional rotations pertaining to the two bonds around the carboxyl group!

15.2.12 Stage 10. Monitoring Structure Solution Progress

The progress of the structure solution can be followed by monitoring the profile c^2 and the difference plot.

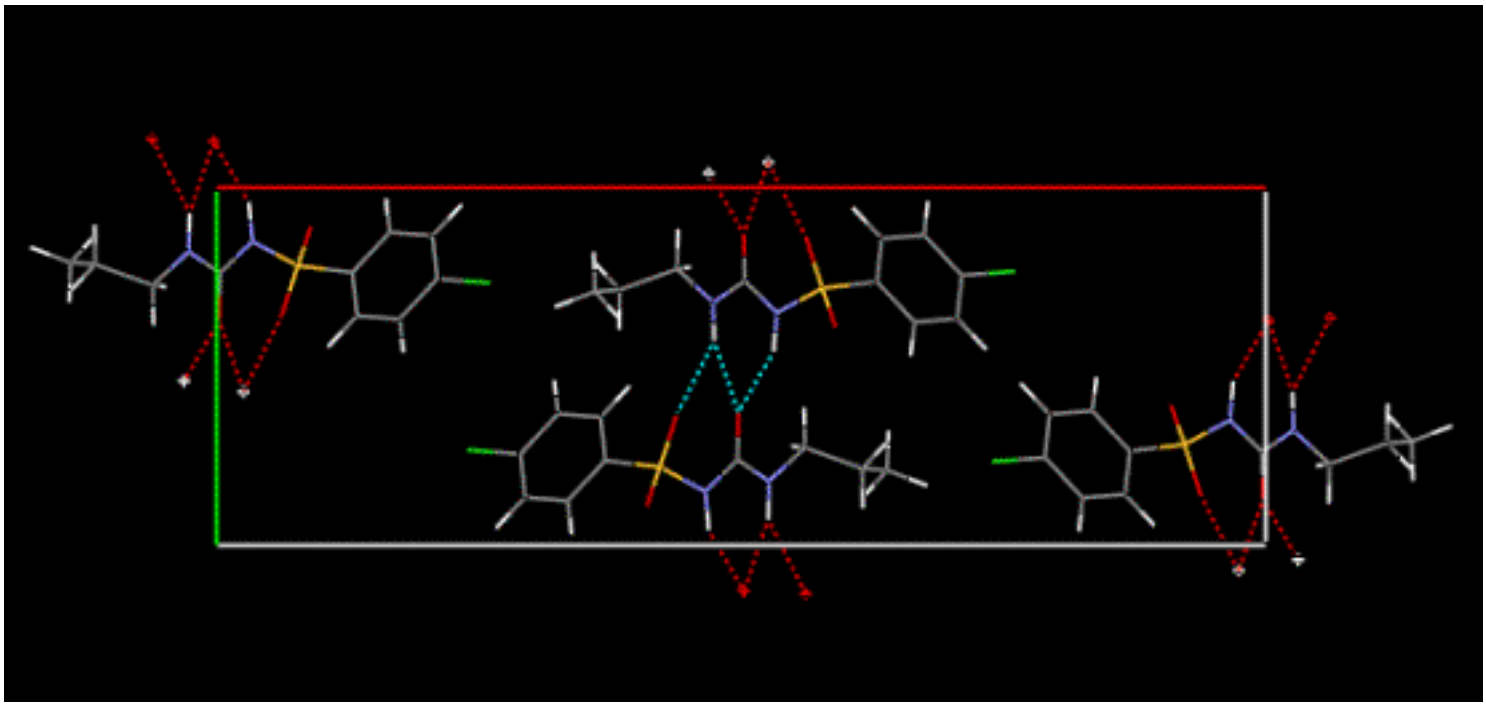
Once a profile c^2 of approximately 10 - 12 or less is reached, you can be sure that a very good structure has been found, as this value is only ~3 times the Pawley c^2 value. Finalise the solution by selecting the **Local minimisation** button and accepting the answer.

If your final profile c^2 is a bit higher than 10, you are clearly close and perhaps only a single atom at the end of the chain is slightly misplaced. Take a close look at the output structure and read the section below.

15.2.13 Stage 11. Examining the Output Structure

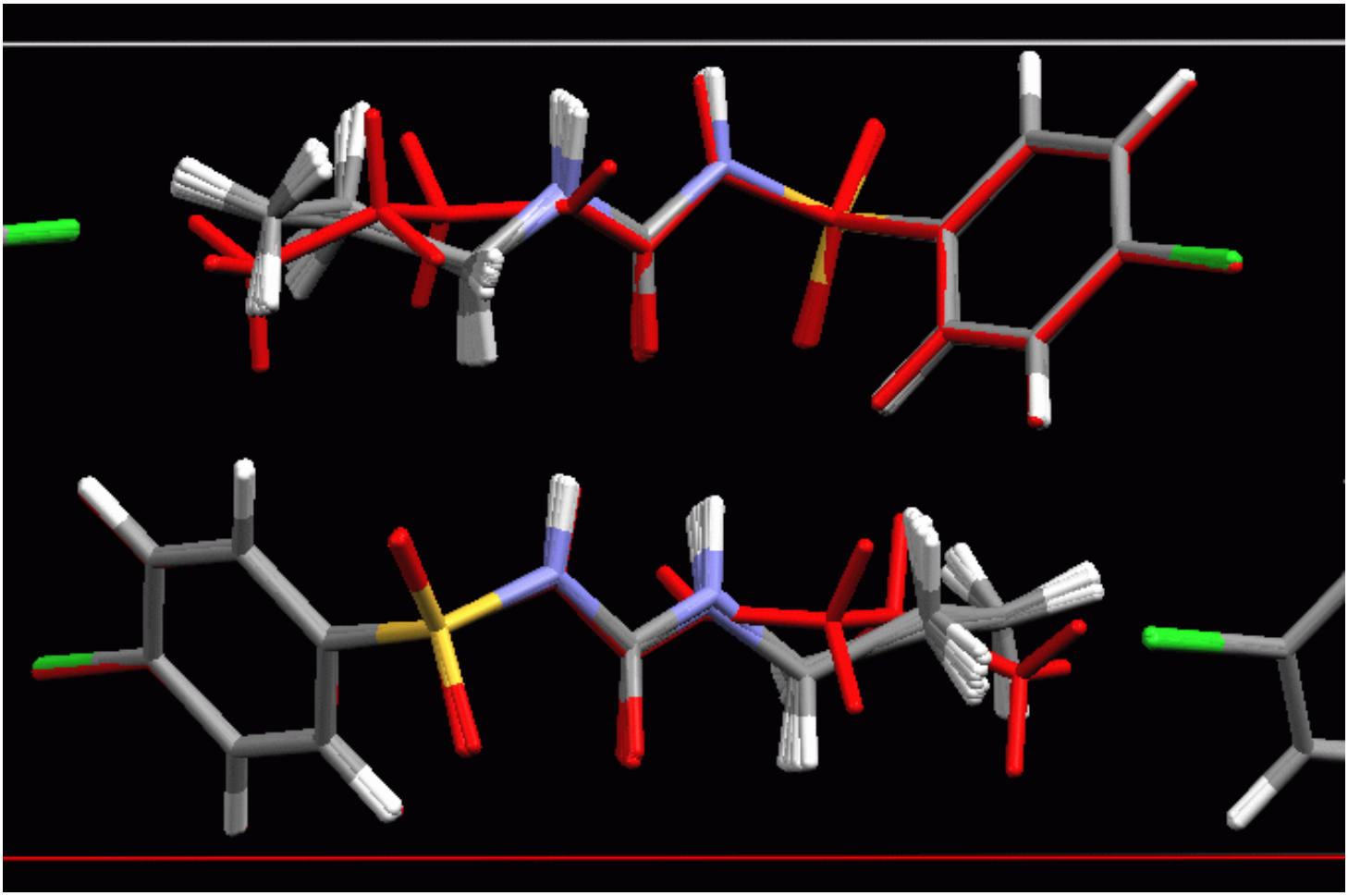
View the structure using the **View** button in the *Simulated Annealing Status* window. The structure should be chemically reasonable in terms of molecular conformation and intermolecular distances. The potential for H-bonding is obvious.

We can examine similar structures in the Cambridge Structural Database, and observe that where there are more acceptors, O, than donors NH, firstly the donors must be satisfied, and secondly, bifurcation of the H-bonds is quite common. This is what we see below, using the Mercury visualiser with Packing and H-bond switched on:



If you have time try doing several SA solution runs and compare the results. This is easy to do in DASH, notice that in the *Simulated Annealing Protocol* window there is the option to start a set of runs, each with a different seed for the random number sequence. If you identify that reasonable termination criteria would be **Max. number of moves / run** = 2,000,000 and Multiplier for Pawley c^2 as 3.5, the runs will terminate either at move number 2,000,000, or when the profile c^2 falls below a value of 3.5 times the c^2 for the Pawley fit. The best solution files are stored in sequence, if you called your run *fit1* the output files are *fit1_001.pdb*, *fit1_002.pdb*, etc.

The accuracy of the solution can be assessed by comparing these independent solutions. An example is given here of an output structure (red) a final profile c^2 of only slightly higher than the lowest c^2 solutions found in a set of runs.



In this case, it is a structure that differs only slightly from the correct structures, corresponding to a local minimum with a profile c^2 only slightly higher than that of the correct crystal structure. The H-bonding scheme is correct, but there are small differences in the terminal side-chain torsion angles.

15.2.14 Stage 12. Applying Modal Torsion Angle Restraints

In the following section the use of modal torsion angle ranges during the Simulated Annealing stage is demonstrated using DASH, and also how this can be facilitated using the CSD System software which now includes *Mogul*. Mogul is a molecular geometry database which forms part of the *CSD System* and is available separately from the *CCDC*.

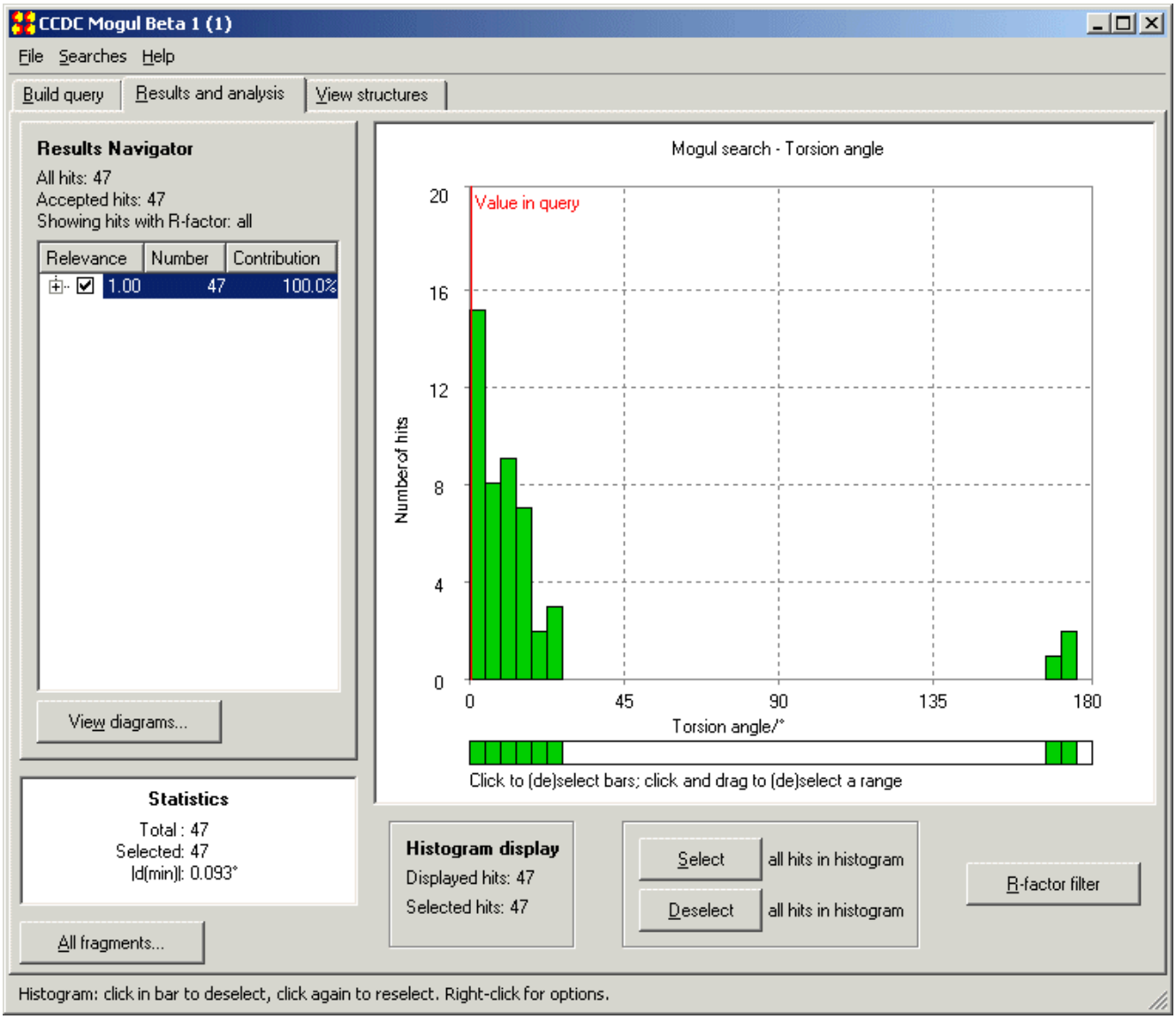
- Press **<Back** (from the Solution Summary dialogue) to return to the introductory Wizard Window. Choose the option **Simulated annealing structure solution**. Reload the z-matrix file for Tutorial 2 and then proceed to the *Parameter Bounds* dialogue box as before.

- Two methods of accessing torsion angle distributions from the CSD are provided:

  Using DASH with Mogul (see page 228)
  Using DASH with the CSD, ConQuest and Vista (see page 231)

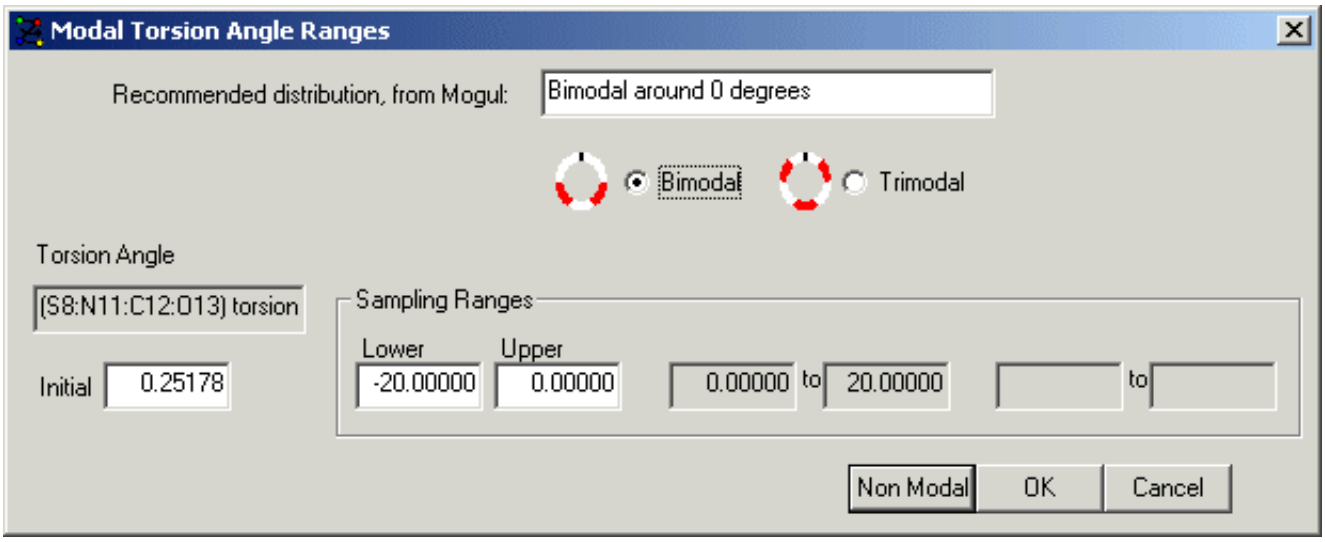
Using DASH with Mogul

- If DASH has access to *Mogul*, the distributions of each torsion angle in the CSD will be examined using Mogul and restricted ranges will be determined from these data will be applied. Scroll down the parameters until the torsion angles are visible.
- The first torsion angle listed in the table is *S8:N11:C12:O13*. Click on the **Modal** button. If the correct path to Mogul is present in the DASH *Configuration* window (access this from the top-level menu by selecting select **Options**, then **Configuration**) then a histogram of the Mogul hits for the selected torsion angle will appear. If a path to Mogul is not present in the *Configuration* window, hit the **Browse...** button in this window and find the location of your installation of the Mogul executable. If a standard installation of Mogul has been performed, DASH should automatically pick up the path to Mogul from the Windows Registry:



- 47 hits for the torsion angle have been found by Mogul, and these can be viewed by clicking on the *View Structures* tab in the Mogul window. If individual bars of the histogram are selected (deselect all hits in histogram and then click on the histogram bars of interest) only these structures are displayed in the *View Structures* window. For example, if the bars around 180^{o} are selected and the structures viewed then Refcodes QERXUK, TOHBUN and TOHBUN01 are displayed.
- Returning to the histogram in the *Results and analysis* pane, it is clear that the torsion angle is most often found to be around 0^{o} with a very small percentage of structures found with torsion angles of 180^{o}. (It should be noted that the Mogul histogram displays all torsion angles, positive and negative on a positive axes, i.e. 0-180^{o}). Close the Mogul window (select **File** from the top-level menu and click on **Exit** in the pull-down menu).

- The *Modal Torsion Angle Ranges* window of DASH will now be displayed. DASH performs a very simple analysis of the distribution of torsion angles returned from Mogul and, if it recognises the torsion angle distribution, will recommend a range of torsion angles to be searched during the simulated annealing; these are displayed in the *Sampling Ranges* section of the window.

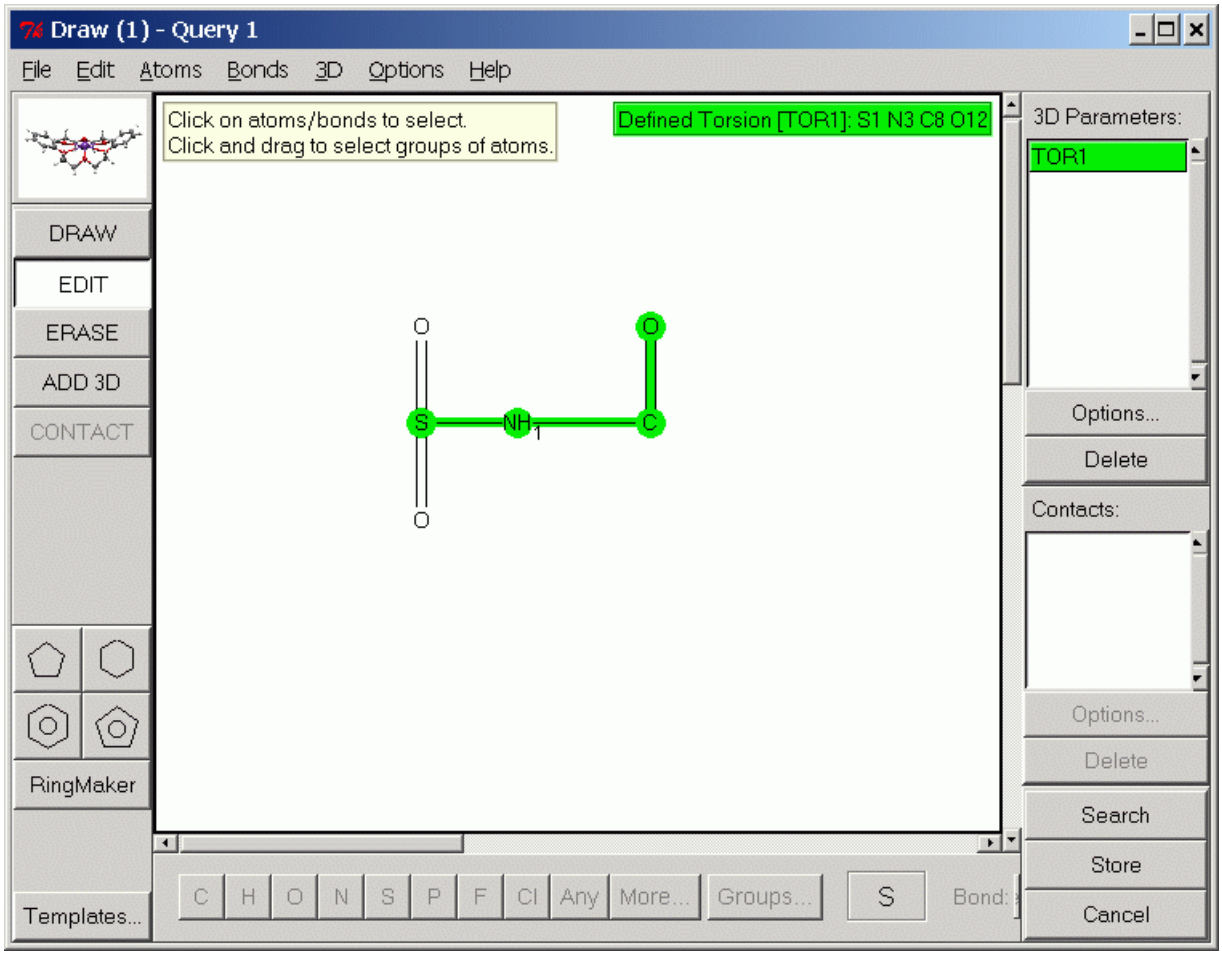


- These ranges are only a recommendation and can be edited and altered. To alter the torsion angle ranges, type the new value in the boxes labelled *Lower* and *Upper*. DASH will calculate the other torsion angle ranges depending on whether the torsion angle has been chosen to be bimodal or trimodal; these ranges are displayed in the grey boxes. To accept the torsion angles displayed click on **OK**. To reject modal torsion angle ranges click on **Non Modal**. Clicking **Cancel** will remove any edits made to the torsion angle ranges since **OK** was last clicked.
- In this case, the suggestion of torsion angle ranges of -20° to 0° and 0° to 20° is appropriate and the ranges suggested by DASH should be accepted by clicking on **OK**. The *Parameter Bound*s dialogue box will be displayed and the torsion angle *S8:N11:C12:O13* will be displayed in red indicating that modal torsion angle ranges have been applied.
- Next click on the **Modal** button for the next torsion angle, *C15:N14:C12:N11*. Again a histogram generated by Mogul will appear and this time it will show a very clear distribution of torsion angles around 180°. When the Mogul window is closed the *Modal Torsion Angle Ranges* dialogue box will be shown with a recommended torsion angle distribution of *Bimodal around 180 degrees*. The torsion angle ranges displayed in the *Sampling Ranges* boxes are satisfactory so click **OK**. This procedure should be repeated for all torsion angle ranges.
- For torsion angle *C4:S8:N11:C12*, the histogram displayed in Mogul shows a cluster of data around 50° to 100°. Upon closing the Mogul window, DASH recommends a bimodal torsion angle range of 45° to 135°. This range adequately covers the distribution returned by Mogul and can be accepted by clicking **OK**. If you wish, the range can be narrowed by editing the *Upper* bounds box.

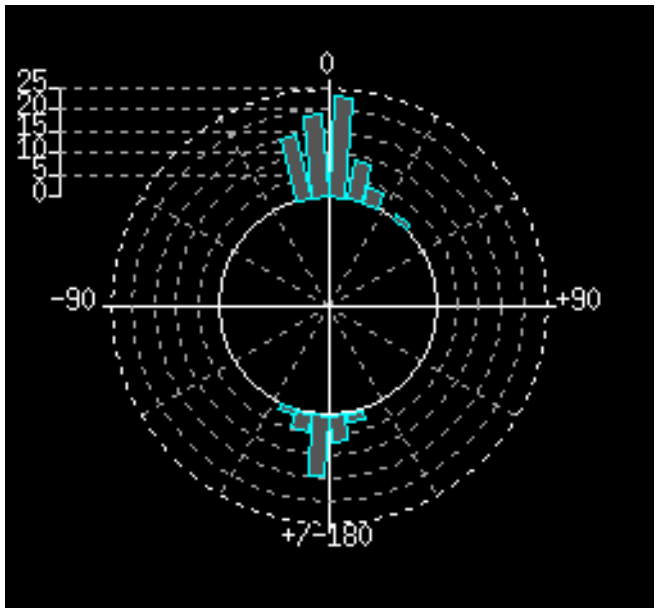
- In the case of torsion angle *C16:C15:N14:C12* DASH cannot process the torsion angle distribution returned from Mogul as it does not recognise the shape of the distribution. Modal torsion angle ranges can either be entered manually in the *Sampling Ranges* boxes (for example a lower bound of 50^o and an upper bound of 180^o could be used) or no torsion angle ranges need be applied. In this case, click the **Non-Modal** button.
- For torsion angle *C3:C4:S8:N11* a bimodal distribution is recommended by DASH, 45^o to 135^o. This covers the majority of the torsion angle distribution returned from Mogul. However, if this range is accepted and **OK** is clicked a warning will pop-up stating that the initial value of the torsion angle is not within the defined ranges. In this case it is acceptable to change the initial value of the torsion angle to, for example 50^o. Clicking **OK** now will apply the torsion angle ranges.
- For torsion angle *C17:C16:C15:N15* the histogram of Mogul shows peaks at approximately 60^o and 180^o indicating a trimodal distribution. Upon closing the Mogul window DASH recommends a trimodal distribution with ranges -150^o to 150^o, 30^o to 90^o and -30^o to -90^o. These ranges are appropriate so click on **OK** to accept them.
- Out of the 6 torsion angles, modal ranges have been set for 5 of them. Proceed through the simulated annealing, as before.
- A simulated annealing run with 10 starts, maximum number of moves 10 million, random seeds 315, 159 was performed with the modal torsion angle ranges recommended by DASH. Of the 10 runs, 8 had a value of profile c^2 below 10 and the average number of moves required was 646 750. A similar run performed without modal torsion angles resulted in 7/10 solutions with a profile c^2 below 10 and the average number of moves required was 1, 828 450.

Using DASH with the CSD, ConQuest and Vista

- If you have access to the Cambridge Structural Database (CSD), Conquest and Vista you can perform the following torsion angle searches for yourself. If not, results for the searches are given. The first torsion angle listed in the *Parameter Bounds* dialogue box is *S8:N11:C12:O13* and it has an initial value of 0.25^o. Draw an appropriate fragment in Conquest and define the torsion angle of interest. A screenshot of a query used is given below:



- By viewing in Vista the torsion angles returned it is clear that this torsion angle is well described by a bimodal distribution at -160 to 160^{o} and -20 to 20^{o}:



- Return to the *Parameter Bounds* dialogue box in DASH, hit the **Modal** button in the row of the *S8:N11:C12:O13* torsion angle. The *Modal Torsion Angle Ranges* dialogue box will pop up and it is here that the determined ranges can be entered:
- In the *Lower* box enter -20.00 and in the *Upper* box enter 20.00. Since the **Bimodal** radio button is active (at the top of the dialogue box) the complementary bimodal range at -160.00 and 160.00^{o} will be determined and displayed. Once you are satisfied that the correct ranges are displayed, press **OK**. This will return you to the *Parameter Bounds* dialogue box and the row of the *S8:N11:C12:O13* torsion angle will be displayed in red, indicating that modal ranges are active.
- Should you wish to define a trimodal torsion angle range, enter the upper and lower bounds of a single range in the *Upper* and *Lower* boxes (for example -160^{o} to 160^{o}). Hitting the **Trimodal** radio button will generate two further torsion angle ranges at +/- 120^{o} from the initial range you have specified (for example at 40 to 80^{o}, and -40 to -80^{o}).
- The following table details the results of searches performed in the CSD v5.24 for all the six torsion angles of this molecule.:

Torsion Angle	Initial value (^{o})	Mode	Modal Ranges (^{o})	Number of Observations
S8:N11:C12:O13	0.25	Bimodal	-160 to 160 and -20 to 20	97
C15:N14:C12:N11	179.72	Bimodal	-160 to 160 and -20 to 20	254
C4:S8:N11:C12	65.08	Bimodal	50 to 90 and -50 to -90	412
C16:C15:N14:C12	-179.37	Cannot define		43
C3:C4:S8:N11	27.90[a]	Bimodal	60 to 120 and -60 to -120	614
C17:C16:C15:N14	-178.54	Trimodal	-160 to 160,b40 to 80 and -40 to -80	71

a. This initial value is determined from a *sketched* model and is reasonably far from the true angle for this structure. If the bimodal ranges indicated are entered and Ok is pressed a dialogue box will pop up and inform you that the initial value does not fall within the defined ranges. To proceed, change the initial value of this torsion angle to for example, 70.00.

- Enter the above torsion angle ranges and start the simulated annealing process.
- In our hands a simulated annealing run started with random seeds 159 and 314 gave 10/10 solutions in an average of 1199250 moves when no restraints were applied. 3/10 solutions had a profile c^2 value below 10.0. With the above restraints applied and therefore the search space reduced, 10/10 runs (starting with the same random seeds) solved and the average number of moves required was 796250. There were 10 solutions found with a profile c^2 below 10.0
- Thus if you have a problem that is proving difficult to solve, with no restraints applied during simulated annealing, it may be valuable to see if there are torsion angle ranges that can be defined (from a search of the CSD) to reduce the search space.

15.2.15 Stage 13. Conclusion

Global optimisation processes may locate local minima, particularly if (a) $Z' > 1$ or (b) the data are of limited resolution. Looking at the above example of a false minimum, it is clear that superficially, they can look chemically sensible. This is hardly surprising, as they lie at a point on the c^2 hypersurface very close to the global minimum of the crystal structure. Accordingly, it is always prudent to run a structure solution multiple times (with different random number seeds) to ensure that a consistent minimum is reached.

15.2.16 References

DICVOL Program:
D. Louer & M. Louer (1972) J. Appl. Crystallogr. ***5****, 271-275.*
A. Boultif & D. Louer (1991) J. Appl. Crystallogr. ***24****, 987-993.*

Model Builder:
WebLabViewerLite Version 3.20 (12/8/98) Copyright 1998 Molecular Simulations, Inc.

Single crystal structure (CSD reference code BEDMIG):
C.H. Koo, S.I. Cho, Y.H. Yeon (1980) *Arch. Pharm. Res.*, ***3***, 37.

Retrieval of Crystallographically-Derived Molecular Geometry Information
Bruno, I.J., Cole, J.C., Kessler, M., Luo, J., Motherwell, W.D.S., Purkis, L.H., Smith, B.R.,Taylor, R., Cooper, R.I., Harris, S.E., Orpen, A.G.
(*in press*)

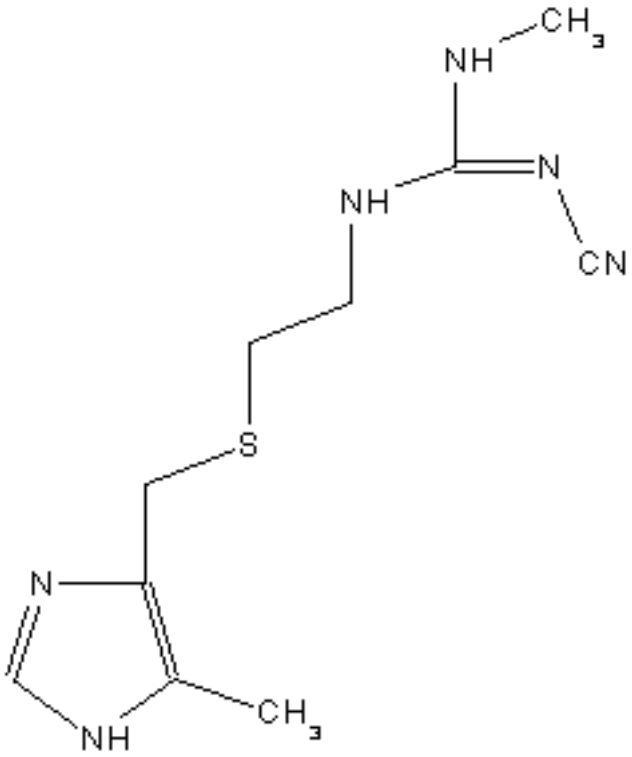
15.3 Tutorial 3 - Handling *cis/trans* Isomerism

15.3.1 Introduction

The object of this tutorial is to guide you through the structure solution of cimetidine, exploring the problems of different molecular models and *cis/trans* isomerism (this tutorial assumes that you have

completed Tutorial 1). In this tutorial, you will learn how to:

- Handle a structure solution where the molecule has different stereoisomers.
- Investigate the success rate with different sources of molecular models.
- Learn a bit more about the Pawley fitting process.



There are possible *cis/trans* stereoisomers, and we will refer to *cis*- when the cyano group is on the same side of the C=N double bond as the sulphur chain.

15.3.2 Data

The data set *Tutorial_3.xye* is a synchrotron X-ray diffraction data set collected on Station 8.3 of the Daresbury SRS. The incident wavelength was 1.5285 Å.

15.3.3 Stage 1: Reading the Data

- Open DASH and select the directory where the data resides.
- Select **View data / determine peak positions** and click **Next >**.
- Select the file *Tutorial_3.xye* using the **Browse...** button.

- Click **Next >**.
- Check that the wavelength and radiation source have been set correctly and click **Next >**.

15.3.4 Stage 2: Examining the Data

The data spans 8.01 to 56.0° 2q. Truncate the data to 2.0 Å resolution and subtract the background using the default window value of 100. You can examine the background curve (green) in detail in the usual way by zooming in on regions of the profile. Since this data has a very uniform low-level background there are no problems, click **Next >**.

15.3.5 Stage 3. Fitting the Peaks to Determine the Exact Peak Positions

Select the first twenty peaks using the method described in Tutorial 1.

Here is a guide to the positions (° 2q) of the first 20 peaks:

9.33	9.97	12.84	13.42	14.20
14.58	16.37	16.57	16.70	17.65
18.27	18.66	18.72	18.93	19.45
19.51	19.72	20.00	20.35	22.92

- Click **Next >**.
- Select **Run>** to run DICVOL or use another indexing program as described in Tutorial 1.

15.3.6 Stage 4. Indexing

If the selected peaks were very close to those given in the previous stage then the DICVOL program returns a monoclinic cell with a = 10.3846 Å, b = 18.7995 Å, c = 6.8201 Å, beta = 106.44°, and volume = 1277.04 $Å^3$ with figures of merit M(20) = 80.4 and F(20) = 198.7. Only one other cell was suggested, also monoclinic with almost identical volume, b and c axes, and alternative a = 10.687 and beta = 111.29°.

15.3.7 Stage 5. Stop and Think

Does the cell make sense? In this case we estimate the molecular volume to be ~320 $Å^3$, from the fact that there are 17 non-Hydrogen atoms in the molecule, each volume approximately 15 $Å^3$ and 16 H each approximately 5 $Å^3$, so (17 x 15 $Å^3$) + (16 x 5 $Å^3$) = 323 $Å^3$. Therefore, given the unit cell volume of ~1277 $Å^3$ we know from this very rough approximation that the cell is most likely to accommodate 4 molecules. At this point, your knowledge of space group frequencies should suggest that $P2_1/c$ is a strong possibility.

15.3.8 Stage 6. Checking the Cell and Determining the Space Group

The space group *P*2 will automatically have been selected. Although the most likely space group is number 14, you should now check through the systematic absences by scrolling through all the space groups, **b**-axis unique, which have $Z = 4$. You will have to decide which setting of space group 14 is correct, $P2_1/a$, $P2_1/c$, or $P2_1/n$. For example, look at the peaks in the region 12 to 15°, $P2_1/n$ creates a tick mark at 13.8 where there is no peak and has no tick mark at 13.42 where there definitely is a peak. $P2_1/c$ also has no tick mark at 13.42 but creates a tick mark at 8.1 where there is no intensity. You should examine other peaks and tick marks to confirm the choice of space group as $P2_1/a$.

15.3.9 Stage 7. Extracting Intensities

Choose 8 isolated peaks from across the pattern (e.g. 9.33, 12.84, 14.58, 17.65, 18.27, 19.72, 23.63, 26.10). Fit these peaks using the method described in Tutorial 1, then carry out the Pawley refinement. The initial 3 cycles of least squares refinement only involve the terms corresponding to the background (which actually has been removed, so notice only 2 polynomial terms are used). This should give a Pawley c^2 of about 76; accept these three cycles. The next 5 cycles of least squares refinement involve the terms describing background, intensities, unit cell and zero point. This should bring Pawley the c^2 down to about 35.

Up to this point the Peak Shape parameters have not been refined. To refine these you fill in the tick boxes **Sigma(size)**, **Sigma(strain)**, **Gamma(size)**, **Gamma(strain)**; it is best to try these options just one at a time. DASH does not allow you to refine both sigma parameters simultaneously, or both gamma parameters simultaneously. It's a good idea to examine the values of the peak shape parameters (**Select Peak Widths** from the **View** menu) and refine only the parameters that have large coefficients, as these are the ones that impact upon the profile fit. In the case of cimetidine, refining just Gamma-strain and setting the number of cycles to 10 rather than the default value of 5, produced a c^2 of 20.0. Your final Pawley c^2 should be in the range 20 - 25.

If the c^2 increased considerably after a refinement, select **Reject** and try refining with a different peak shape parameter.

Accept your best Pawley fit, making a note of the c^2, and save it as *Tutorial_3.sdi*.

15.3.10 Stage 8. Molecule Construction

Construct a 3D molecular description of the molecule using your favourite modelling software and save it in pdb, mol or mol2 format. If you do not have a model building program to hand, there is a file supplied with the tutorial, *Tutorial_3-cis.mol2*. (This model for the cis-isomer was created using the Spartan program using default minimisation settings.) Select the file *Tutorial_3-cis.mol2*.

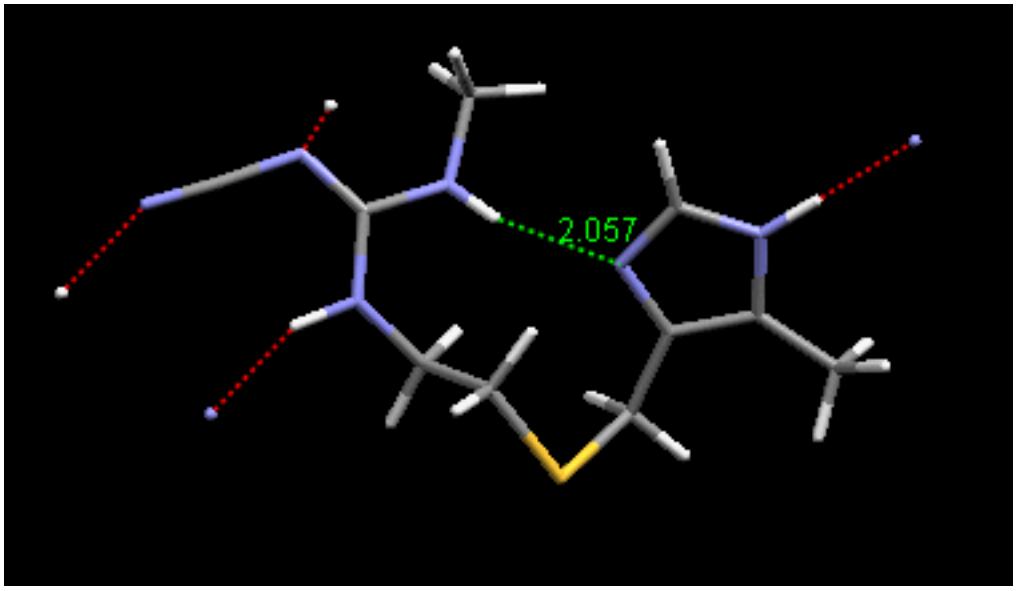
15.3.11 Stage 9. Setting up the Structure Solution Run

- Start DASH as before and select **Simulated annealing structure solution** from the Wizard.
- Select the *Tutorial_3.sdi* file.
- Click on the  icon and select *Tutorial_3-cis.mol2* (the file that you created in Stage 8); a Z-matrix file called *Tutorial_3-cis.zmatrix* will be generated automatically.
- Read in the *Tutorial_3-cis.zmatrix* file.
- At this point DASH will confirm that there are 14 independent parameters. These parameters are listed when you click on **Next >**. There are 3 parameters describing the positional coordinates, 4 (of which 3 independent) describing the molecular orientation within the unit cell and 8 variable torsion angles. Note that in this model we are keeping the *cis*-configuration fixed.
- Click **Next >** to proceed to the *Simulated Annealing Protocol* window. Leave the parameters set at the default values, click **Next >** again, then click **Solve >**. This will take a little longer than the earlier Tutorial examples as there are more torsion angles allowed to vary: 8 compared with 6 in Tutorial 2.

15.3.12 Stage 10. Monitoring Structure Solution Progress

The progress of the structure solution can be followed by monitoring the profile c^2 and the difference plot.

At some point in the run you should see a dramatic fall in the c^2 value from about 1000 to around 200. At this point you can investigate if a local minimisation produces an improvement - the answer will almost certainly be 'Yes', so accept this improved point. Have a look at the structure with the **View** button. You should see that the H-bonding of groups is now quite plausible; always look first for unsatisfied H-donor atoms. You will see also that the molecule has coiled around to form an intra-molecular H-bond from the NH near the end of the chain to the acceptor N on the imidazole ring.



15.3.13 Stage 11. Examining the Output Structure

View the structure using the **View** button in the *Results from Simulated Annealing* window. All should look reasonable, there should be no abnormal close contacts between molecules, except perhaps for some H-atoms. The H-atoms contribute such a small percentage of the total scattering power of the molecule that they have very little effect on the value of c^2. The positions of methyl H atoms in particular are poorly determined, as they have been placed in calculated positions and not allowed to rotate. There is a crystal structure for this *cis*-isomer in the CSD (CIMEDT03); the H-bonding scheme matches this exactly. There may appear to be an extra H-bond to the cyano-N in your solution, but this will be just within the arbitrary limit set for distance scanning for H-bonds in the visualiser. (The Mercury visualiser allows you to easily examine the H-bond network just by clicking on the H-bonds.)

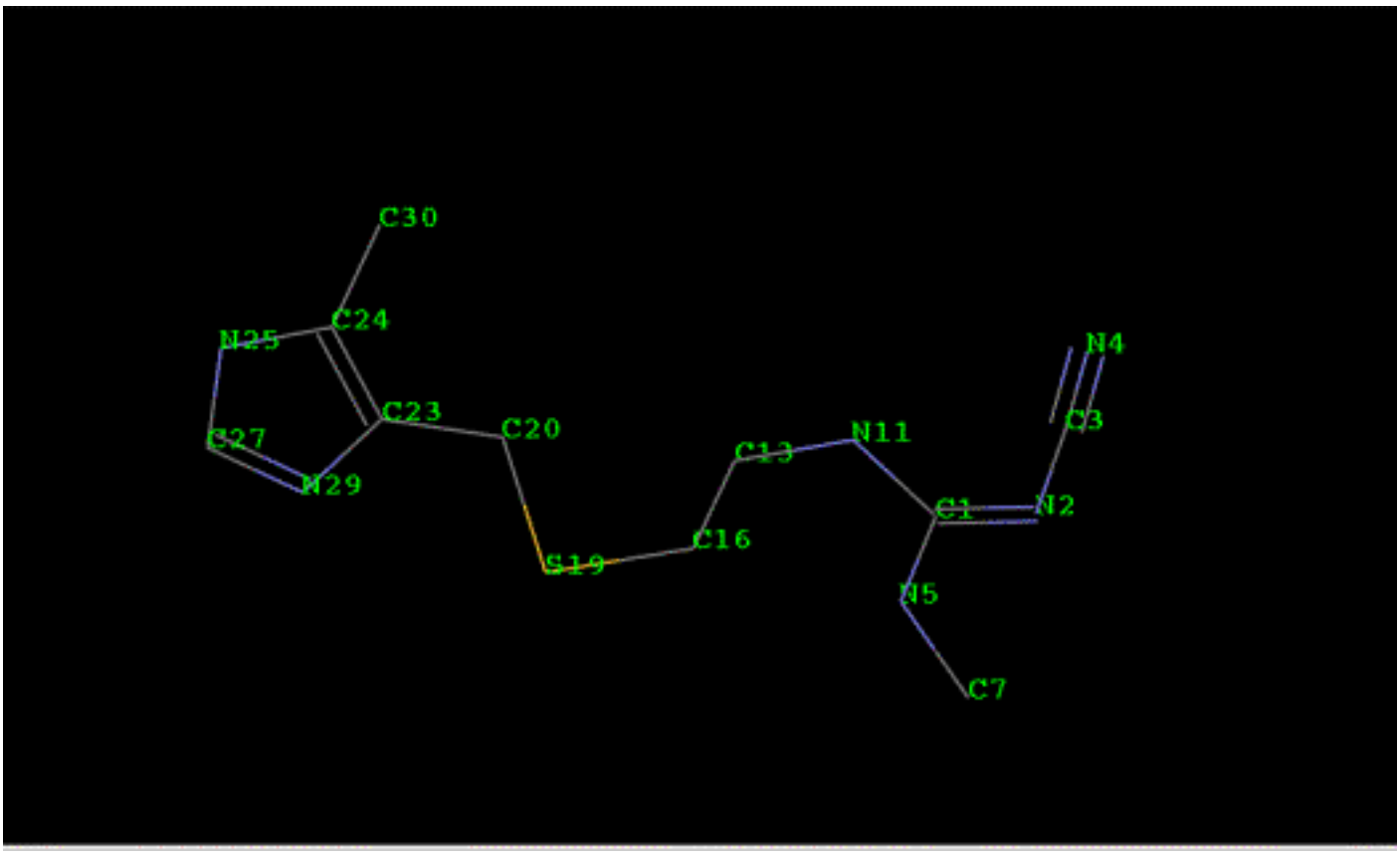
15.3.14 Stage 12. Experiments Altering the cis/trans Isomer

It is interesting to do the experiment of trying to solve the structure with the *trans*-isomer. You can either build a model with the *trans*-configuration, save it as *Tutorial_3-trans.mol2* (a *trans*-isomer model file is provided with this Tutorial as *Tutorial_3-trans.mol2*), or use a more advanced feature of DASH to allow the model to rotate about the relevant C=N double bond.

You will find that if you take a *trans*-isomer model the SA solution process will not get very far. Using the multiple-run feature of DASH a typical set of 5 runs had final c^2 of 367, 452, 647, 975, 624, with hopelessly tangled close contacts.

You can modify the previous *cis*-cimeditidine model to cover both *cis*- and *trans*- configurations. To do this you need to make one small change to the Z-matrix file, *Tutorial_3-cis.zmatrix*. Look at the

labelling of the source molecular model provided with this Tutorial, *Tutorial_3-cis.mol2*, shown below, hydrogens omitted.



- The relevant torsion angle we now want to vary is C3-N2-C1-N11. If you examine the Z-matrix you will see the line that specifies this torsion angle, looking at the labels on the right, count up 15 from bottom. The torsion angle (column 6) is set at 1.4448765; the number 0 that follows is a control indicator to tell DASH that this is a fixed torsion angle; if you change this to 1 this indicates a variable torsion angle.
- Make this change and save your Z-matrix as a new file, e.g. *cistrans.zmatrix*. Now begin a structure solution run loading this new matrix; look down the list of parameters in the *Parameter Bounds* window. This angle is now freely variable from 0 to 360°, and the total DASH parameter count is now 15. Go to the *Simulated Annealing Protocol* window, where there is the option of performing multiple runs. If you do a set of 5 runs as before, with the maximum number of moves per run 3,000,000 and c^2 multiplier 3.0, you will get probably about 2 or 3 correct solutions out of 5.
- A typical set of runs gave c^2 = 329, 200, 76, 199, 74. The low values 74 and 76 are very satisfactory solutions; with variable torsion angle C3-N2-C1-N11 values around -6.0°, which is the *cis*-conformation.

(The Z-matrix format is described in Appendix F of the DASH User Guide.)

15.3.15 Stage 13. Effect of Molecular Models on Simulated Annealing

Another interesting experiment is to see how much the fine detail of the model building affects the chance of solution with DASH. Experiments were carried out with three models, the files are provided with this Tutorial as *Tutorial_3-ModelA.zmatrix, Tutorial_3-ModelB.zmatrix, Tutorial_3-ModelC.zmatrix*.

- Model A was prepared using the ISIS/Draw sketcher and WebLabViewer, with no energy minimisation, exported as a mol file.
- Model B was prepared using Spartan to sketch and then do a simple energy minimisation, exported as a mol2 file.
- Model C was the CIMETD03 structure taken from the CSD as a mol2 file. H-atom positions were recalculated at ideal geometry using Rpluto.

Each model was used for a set of 30 SA runs, with **Max. number of moves / run** 3,000,000 and **Profile chi-sqd multiplier** 3.0. The Pawley-fit c^2 was 22.06. The solutions had profile c^2 as follows:

A 18 x ~149*, 11 x ~282, 1 x 443
B 19 x ~103*, 11 x ~241
C 20 x ~83*, 10 x ~268

The solutions marked * are correct - with good H-bond patterns, and torsional geometry close to the CSD. This gives us some confidence that the solution of structure with 8 torsion angles can be carried out with a good likelihood of success. It is interesting that Model A, which had not been subjected to energy minimisation, still gave correct solutions, but with a higher c^2 than the other solutions. You should try a multiple run with your own constructed *cis*-isomer model file.

15.3.16 Stage 14. Rietveld Refinement

In order to demonstrate the utility of the built-in rigid-body Rietveld refinement module, a refinement on simulated annealing solutions generated from Model B will be outlined. A similar process could be carried out using one of the interfaces to an external refinement package. Model B was generated in PCSpartan Pro and a simple energy minimisation performed and therefore can be expected to have bond angles and bond lengths that are only roughly in agreement with the crystal structure values.

- Carry out multiple simulated annealing runs with the molecular model described by *Tutorial_3-ModelB.zmatrix* and random seeds 314 and 159. Once the simulated annealing is complete and the *Analyse Solutions* dialogue box is displayed, click on the **Rietveld** button corresponding to the best solution.
- The refinement of the best solution from these simulated annealing runs should take the structure

towards the solutions obtained for a refinement on Model C, the model generated from a single crystal structure.

- In the runs performed here, the best solution had a profile c^2 of 52.88 and an intensity c^2 of 41.70. After allowing all the sets of parameters to refine, individually (for example Global isotropic temperature factor, then torsion angles, then angles, then bond lengths and zmatrix) the profile c^2 and intensity c^2 had reduced to 34.48 and 21.14, respectively.
- The following table lists selected angles in Model B solutions before and after Rietveld refinement. The values found in the single crystal structure are also given for comparison. It can be seen that the solution for Model B is moving towards the single crystal structure during Rietveld refinement.

Angle	Before RR (^{o})	After RR (^{o})	Crystal Structure (^{o})
N11:C1:N5	111.53	117.54	123.89
N11:C1:N2	127.43	124.36	117.52
N2:C3:N4	176.98	170.13	170.34
N25:C24:C30	129.70	120.79	121.81
C20:S19:C16	96.47	99.07	105.50

- Not many refinement cycles are performed before changes in the c^2 values become very small. Like Tutorial 1, small changes can be brought about in the value of intensity c^2 by repeatedly refining the bond angles and bond lengths for example. However, given the resolution of the data, these changes do not represent an improved set of coordinates for this structure.

15.3.17 Stage 15. Conclusion

- One can clearly distinguish between *cis*- and *trans*- isomers in this case.
- It is possible to use DASH to allow *cis/trans* as a variable torsion angle, giving the correct solution.
- The accuracy of the molecular model does matter.

15.3.18 References

DICVOL Program:
D. Louer & M. Louer (1972) J. Appl. Crystallogr. ***5****, 271-275.*
A. Boultif & D. Louer (1991) J. Appl. Crystallogr. ***24****, 987-993.*

Model Builders:
WebLabViewerLite Version 3.20 (12/8/98) Copyright 1998 Molecular Simulations, Inc.
PC Spartan Pro Version 1.0.5 (16/8/2000) Copyright (1996-2000) Wavefunction, Inc.

Single crystal structure cis-cimetidine (CSD reference code CIMETD03):
R.J. Cernik, A.K. Cheetham, C.K. Prout, D.J. Watkin, A.P. Wilkinson, B.T.M. Willis, (1991) *J. Appl. Crystallogr*. **24**, 222-226.

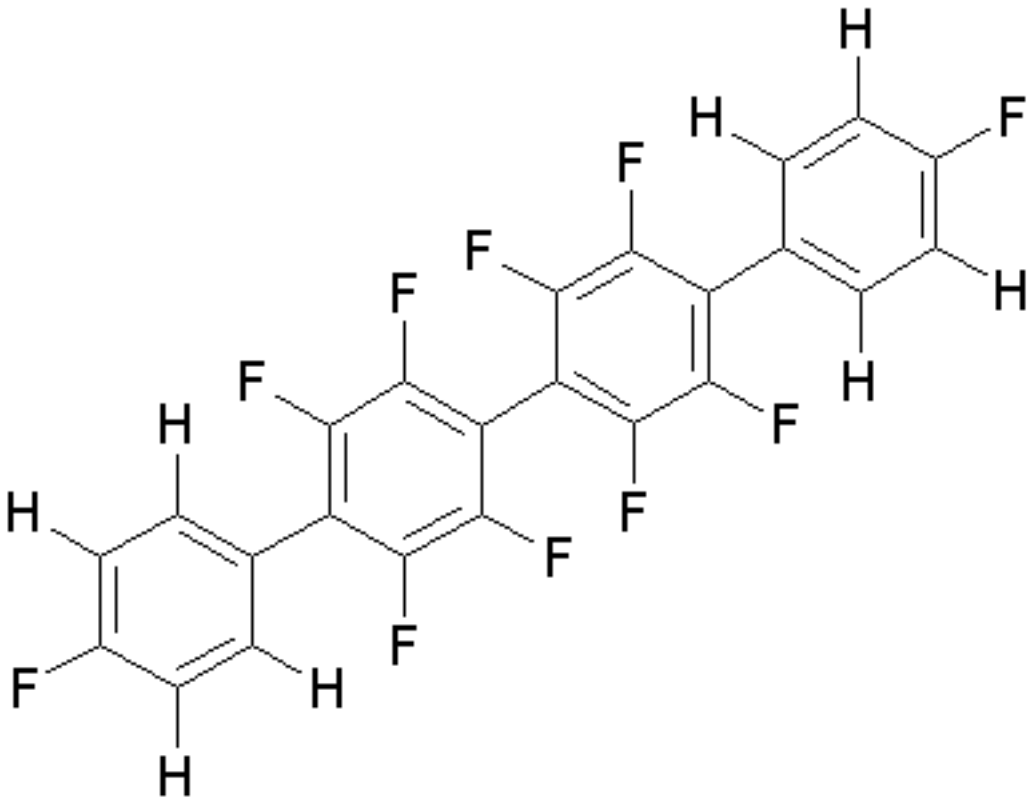
Single crystal structure trans-cimetidine [CSD reference code CIMETD01].
L. Parkanyi, A. Kalman, B. Hegedus, K. Harsanyi, J. Kreidl (1984) *Acta Crystallogr.* C**40**, 676-679.

15.4 Tutorial 4 - Handling a Structure in Which There is a Space Group Ambiguity

15.4.1 Introduction

The object of this tutorial is to guide you through the structure solution of decafluoroquaterphenyl (DFQP). It assumes that you have completed the previous tutorials. In this tutorial, you will learn how to

- Handle a structure solution in which there is a space group ambiguity.
- Deal with a structure that has a potential centre of symmetry.
- Deal with a more prominent background than you have encountered so far.
- Handle a difficult Pawley fitting problem.



15.4.2 Data

The data set *Tutorial_4.xye* is a laboratory X-ray diffraction data set collected by Dr. Lubo Smrcok. The incident wavelength was 1.789 Å.

15.4.3 Stage 1: Reading the Data

- Open DASH and select the directory where the data resides.
- Select **View data / determine peak positions** and click **Next >**.
- Select the file *Tutorial_4.xye* using the **Browse...** button.
- Click **Next >**.
- Check that the wavelength and radiation source have been set correctly and click **Next >**.

15.4.4 Stage 2: Examining the Data and Removing the Background

The data spans 4 to 61° 2q. Remember that this data has been collected at a relatively long wavelength and so the real-space resolution of the data is only ~1.8 Å. Truncate the data to 2.0 Å resolution.

Having examined the data, we really want to strip out the background. This is because the data, whilst good, are nowhere near as good as the synchrotron data sets that you examined in Tutorials 1 and 2. If we defer background modelling until the Pawley fit stage, we have an additional set of parameters to worry about at that stage. With the weaker data at higher angles, there is always a chance that correlations between the weak peaks and the background parameters may cause instabilities in the fit. These can be avoided by removing the background at this stage.

Select **Preview** using the default value of 100 for the filter window size. Examine the background fit carefully at all points in the pattern, but especially at the low and high angle regions. The fit is excellent, with only a marginal underestimation at low angle. Try altering the window size to 50 and select **Preview**. Note the better fit at low angle, and also the increased flexibility in the background shape that decreasing the window size has brought about. Although you could proceed with either value (as both give an excellent fit) return to the less structured background by changing the window size back to 100 and click **Apply** to strip off the background. The background-subtracted pattern is displayed. Examine it closely before proceeding to the next stage.

15.4.5 Stage 3. Fitting the Peaks to Determine the Exact Peak Positions

Select the first 22 peaks using the method described in Tutorial 1.

Here is a guide to the positions (° 2q) of the first 22 peaks:

8.7661	17.3011	18.7498	20.7870	21.3166
21.5902	23.6311	24.4517	24.9854	25.4535
27.1252	27.7534	28.1742	29.1876	30.7017
33.5830	33.7804	34.1816	34.7082	34.9715
35.2415	35.4262			

- Click **Next >**.
- Select **Run>** to run DICVOL or use another indexing program as described in Tutorial 1.

15.4.6 Stage 4. Indexing

Your indexing program may reveal a number of possible unit cells. The unit cell with the highest figures of merit should be monoclinic with volume ~1794 Å^3. The DICVOL program returns a monoclinic cell with a = 24.04678 Å, b = 6.15668 Å, c = 12.42973 Å and beta = 102.753°, Volume = 1794.80 Å^3 with figures of merit M(22) = 16.1 and F(22) = 29.3. Whilst not fantastic figures of merit, we can note that there are nearly 100 calculated peaks for this cell, as against the 22 that were input. This might indicate that there are a lot of systematic absences, or it may indicate that the cell is

wrong.

15.4.7 Stage 5. Stop and Think

Does the cell make sense? In this case, given that the molecule may well adopt a planar configuration, it is difficult to estimate the likely molecular volume. Assuming 4 molecules per cell and dividing 1800 $Å^3$ by 4, we get 450 $Å^3$, which is certainly enough to accommodate the molecule's backbone of 24 carbon atoms (15 $Å^3$) + 10 fluorines (10 $Å^3$) = 460 $Å^3$. So the cell is worth checking.

15.4.8 Stage 6. Checking the Cell and Determining the Space Group

It is clear that there are a great many excess tick marks, indicating probable systematic absences, this means that the space group must be of substantially higher symmetry than *P*2. Zoom into the 10 - 16° region of the pattern and watch the correspondence between the tick marks and the observed reflections as you scroll through some of the possible space groups. You will see that many of the space groups can be ruled out immediately, for example, the primitive cells predict many peaks that are not observed. A centred cell is therefore likely, and so *C*2/*c* is a likely choice (see Appendix D in the DASH User Guide). Select this group and examine the pattern closely. Things look good at low angle but the peak at ~24.5° is misplaced.

Altering the setting to *I*2/*a* results in excellent agreement throughout the pattern, so this appears to be the best choice. Note, however, that *Ia* has the same systematic absences as *I*2/*a* and therefore gives exactly the same level of agreement. Using the table in Appendix D of the DASH User Guide, the centrosymmetric space group *I*2/*a* (*C*2/*c*) is about 7 times more common than the non-centrosymmetric space group *Ia* (*Cc*). As the molecule possesses a molecular centre of symmetry in the middle of the bond between the two central rings, *I*2/*a* is certainly the more likely choice.

15.4.9 Stage 7. Extracting Intensities

Pawley fitting this pattern in either *I*2/*a* or *Ia* will give identical results (the absences are the same) and so we will fit *Ia*. We want to delete the last group of 3 peaks as they are highly overlapped, in the region of 35°, (sweep this range and select the **Delete** key). The program now detects that it has peaks available for unit cell refinement and so the *Pawley Refinement Status* window appears automatically, as the peaks widths for all the indexing peaks that you fitted earlier are still available to DASH.

Select **Refine**. The initial 3 cycles of least squares refinement only involve the two terms corresponding to the linear background and to the individual reflection intensities; accept these three cycles. Using the cell constants listed in Stage 4, the Pawley c^2 is about 2.7. Proceed and refine the unit cell and zero-point along with the background and the intensities. You may get a warning from DASH stating that errors have been detected due to instabilities in the Pawley fit. If so, reject the fit and increase the **Overlap criterion** to 2.0. Select **Refine** and you should get a stable refinement with a Pawley c^2 of about 1.9.

Accept your best Pawley fit and save it as *Tutorial_4.sdi*.

15.4.10 Stage 8. Molecule Construction

Construct a 3D molecular description of the molecule using your favourite modelling software and save it in pdb, mol or mol2 format. This can be done, for example, by importing an ISIS/Draw sketch into WebLabViewer (for further details, see Tutorial 1). Save this as *Tutorial_4-full.pdb, Tutorial_4-full.mol* or *Tutorial_4-full.mol2*. The remainder of this tutorial is based upon a Z-matrix constructed importing the above ISIS/Draw sketch of the molecule into WebLabViewer and exporting it as a mol file. (If you do not have a model builder to hand there are files provided with the tutorial: *Tutorial_4-full.mol2* and *Tutorial_4-half.mol2*.)

15.4.11 Stage 9. Setting up the Structure Solution Run

- Continue on from the Pawley fitting stage by pressing **Solve >**.
- Click on the  icon and select *Tutorial_4-full.mol2* (the file that you created in Stage 8); a Z-matrix file called *Tutorial_4-full_1.zmatrix* will be generated automatically.
- Read in the *Tutorial_4-full.zmatrix* file, which has three moveable torsion angles.

At this point DASH will confirm that there are 9 independent parameters. These parameters are listed when you click **Next >**. There are 3 parameters describing the positional co-ordinates, 4 (3 of which independent) describing the molecular orientation within the unit cell and 3 variable torsion angles. All **F** boxes are unticked by default, indicating that all 10 parameters are allowed to vary during structure solution. Click **Next >**, leave the parameters set at their default values, click **Next >** again, then **Solve >**; the simulated annealing process begins.

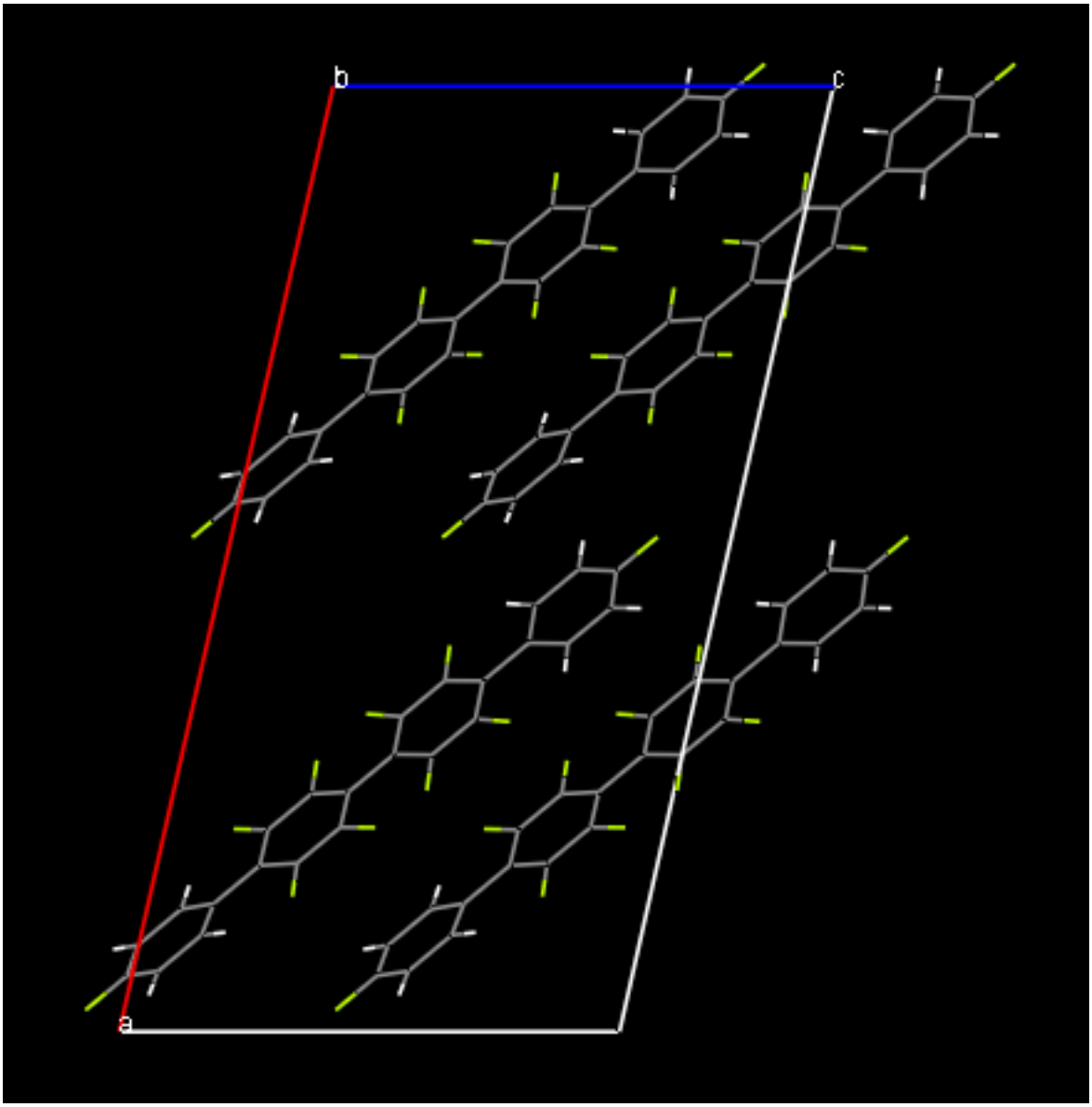
15.4.12 Stage 10. Monitoring Structure Solution Progress

The progress of the structure solution can be followed by monitoring the profile c^2 and the difference plot.

The profile c^2 should fall fairly quickly to below 20 and the fit to the data should look not bad, with the residual misfits distributed throughout the pattern.

15.4.13 Stage 11. Examining the Output Structure

View the structure using the **View** button in the *Results from Simulated Annealing* window. The molecular conformation and the packing look reasonable. However, we have still only explored *Ia*.



15.4.14 Stage 12. Exploring the Possibility of *I*2/*a*

A: Space Group *Ia*

There is a quick and easy way to explore whether or not the true space group is *Ia* or *I*2/*a* whilst performing all SA runs in *Ia* In the previous run, the centre of mass of the molecule was allowed to roam freely throughout the unit cell. If the space group truly is *I*2/*a*, then the centre should lie either on the origin or on the 2-fold axis. Accordingly we can:

- Constrain the centre of mass to lie at 0,0,0
- Constrain the centre of mass to lie on 0.25,y,0

and repeat the structure solution runs in *Ia* to see the fits that are obtained.

How to constrain the molecule to lie on special positions

- To constrain the centre of the molecule to lie on the origin of the cell, stop the current annealing run by pressing **Stop**, and return to the *Parameter Bounds* window.
- Enter values of *0.0* for the initial values of *x(frag1)*, *y(frag1)* and *z(frag1)* and then click the **F** check box for each of these variables in order to fix the x,y,z position of the molecule within the unit cell at the fractional co-ordinates *0,0,0*. Note that by default, DASH uses the centre-of-mass of the molecule as the x,y,z reference point, and for the DFQP molecule, this corresponds to the midpoint of the central bond. You can now proceed with the simulated annealing run knowing that the centre-of-mass of the molecule will always be constrained to lie at *0,0,0*.
- Similarly, for the SA in which we wish to hold the centre-of-mass on the 2-fold axis, return to the *Parameter Bounds* window. Following the same procedure as just outlined, fix *x(frag1)* at 0.25, leave *y(frag1)* to vary and fix *z(frag1)* at 0.0.

Fixing the centre of mass at 0,0,0 causes the structure solution to *stick* at a very high profile c^2, around 130. In contrast, after constraining the centre of mass of the molecule to lie on the 2-fold axis, the profile c^2 falls rapidly to around 20 and local minimisation reduces this still further to around 18. The packing motif is identical to that obtained in *Ia*.

Note that a 2-fold rotation of the molecule about the **b**-axis does not give an exact mapping from one half of the molecule to the other, as in *Ia*, there is no constraint upon the torsion angles to produce this. However, it is so close to doing so that it is safe to conclude that the molecule crystallises in space group *I*2/*a* and that a Rietveld refinement with only half a molecule in the asymmetric unit will be successful.

B: Space Group *I*2/*a*

You can (if you want) re-fit the diffraction data using the same unit cell and selecting space group *I*2/*a* but there is in fact an easier way of running the SA in *I*2/*a*. As stated previously, *Ia* and *I*2/*a* have the same systematic absences and so Pawley fitting in either space group will give the same result. Accordingly, we can use the Pawley fit files already created and simply modify the *Tutorial_4.sdi* file to inform the SA that we now wish to solve in *I*2/*a*. Copy *Tutorial_4.sdi* to *Tutorial_4-half.sdi* and open the file in a text editor. Look for the following line:

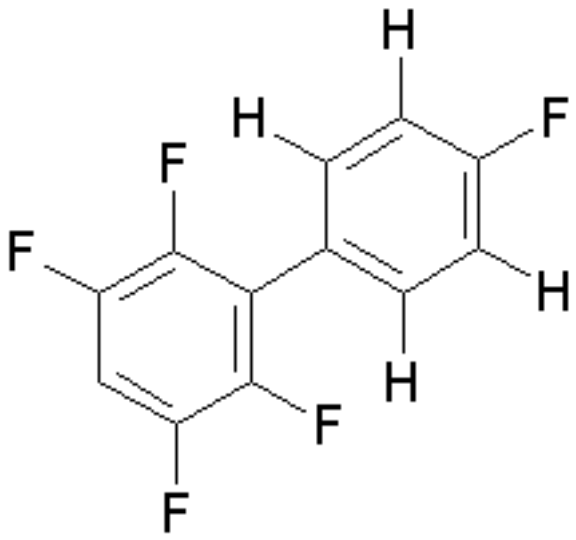
SpaceGroup 52 9:b3 I 1 a 1

and change it to:

SpaceGroup 69 15:b3 I 1 2/a 1

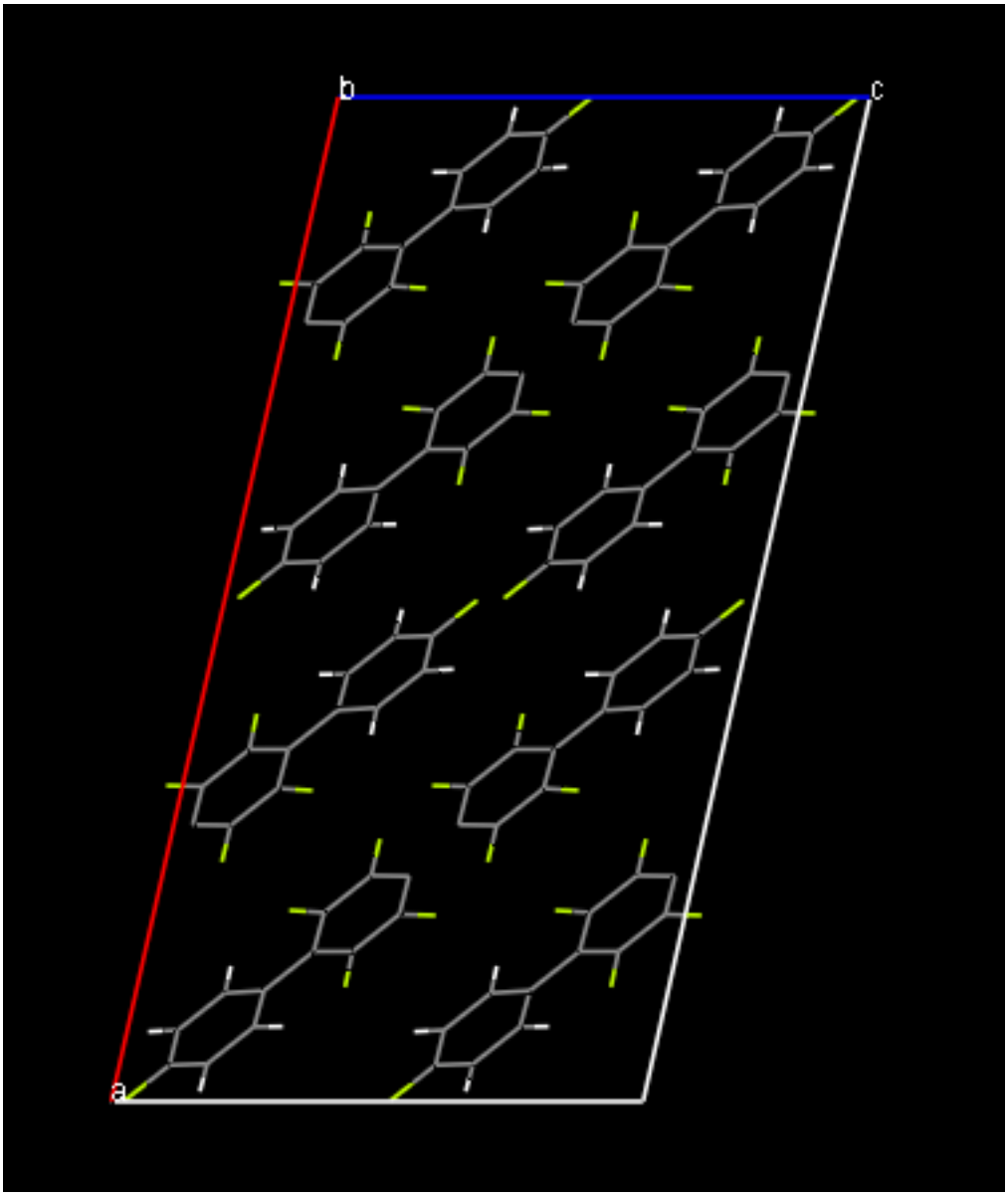
and then save the file. You have now told the program that the SA must now be performed in space group *I*2/*a* (consult the DASH User Guide, Appendix D.3 for an explanation of the format of the Space Group line), whilst leaving the pointers to the existing Pawley fit files.

Next, as we are now in *I*2/*a*, we only require half a molecule to fill the asymmetric unit.



Construct a *Tutorial_4-half.mol* file based on the above diagram and read it into DASH. The resultant *Tutorial_4-half_1.zmatrix* file only has a single torsion angle - the torsion angle between the two 'halves' of the full molecule is automatically determined by the orientation of the molecule within the unit cell.

Using the Wizard, load the new *Tutorial_4-half.sdi* file and the *Tutorial_4-half_1.zmatrix* file. Do not fix any of the variable parameters (i.e. allow the molecule to roam the unit cell) and start a SA run. The profile c^2 should fall rapidly to around 20. Viewing the molecule shows that the space group symmetry is indeed constructing the whole molecule (though the central bond is not displayed on-screen the distance C-C can be measured to be about 1.6 Å).



There is therefore no doubt that this molecule crystallises in space group *I*2/*a*. Note that the molecule is sitting on a 2-fold rotation axis and not the centre of symmetry.

15.4.15 Stage 13. Conclusion

This tutorial has shown that there are several ways to solve the crystal structure of DFQP using global optimisation, all of them equally valid. Structure solutions of this complexity using DASH take so little time to execute that it is worth investigating the various possibilities in order to be certain that you have the correct answer.

The final fit to the data is not that great, but the chemical sense of the structure is such that there is no doubt that the structure is correct. The published Rietveld refined structure (Smrcok et al.) for this molecule confirms this. Accordingly, note that it is entirely possible to obtain a profile c^2 that is a factor of 10 higher than the Pawley c^2 and still have the correct structure.

Some remarks on Rietveld Refinement are in order. The published structure reported the results of an unrestrained Rietveld refinement, which shows quite severe distortion of the benzene rings. This is a natural consequence of allowing too many variables to be optimised against the rather limited data, especially this set of laboratory data of lower accuracy than synchrotron. A tradition has grown up of allowing unrestrained refinement of all atomic positions in order to *prove* that the crystal structure is correct. This certainly proves that the atoms all fit well with the low resolution electron density represented here by only 174 reflections, which are extracted by Pawley fit corresponding to the complete data set in 2q, corresponding to 1.763Å resolution. However a more realistic model for the real crystal structure is obtained if one uses the DASH Rigid-group Rietveld refinement.

It has be seen in the previous section (12) that the low-resolution data gives an unreasonably long value for the central C-C bond, 1.60Å, when we refine with a half-molecule in *I2/a*. A better model for the full crystal structure is to use the constrained full molecule placed with its centre of mass on the crystallographic 2-fold axis at (0.0, y, 0.25). If the DASH Rietveld refinement is applied to the data available to 1.763Å resolution, in space group *Ia*, we obtain typical solutions with Chi-sqd of about 86 and Profile Chi-sqd of 11.3. An example refinement of the global isotropic temperature factor scale, followed by refinement of Translations, (y only), and Rotations gave values of 0.6717, and Chi-sqd of 85.87, 11.30. A check of the shortest inter-molecular contacts shows shortest C...H 2.60Å, F...F 2.56Å, and H41...F16 2.09Å. This latter value is rather closer than expected being 0.5Å shorter than the van der Waals radii sum, but the other short-contact values can be seen in CSD single crystal structures.

15.4.16 References

DICVOL Program:
D. Louer & M. Louer (1972) J. Appl. Crystallogr. ***5****, 271-275.*
A. Boultif & D. Louer (1991) J. Appl. Crystallogr. ***24****, 987-993.*

Model Builders:
WebLabViewerLite Version 3.20 (12/8/98) Copyright 1998 Molecular Simulations, Inc.

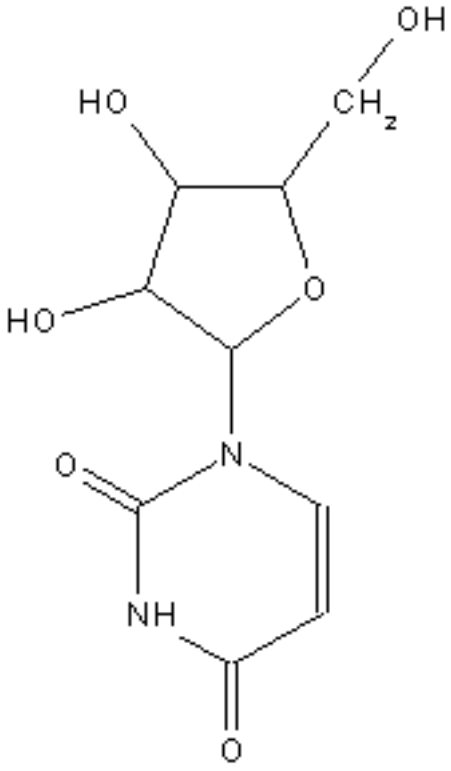
Crystal structure of decafluoroquaterphenyl:
L. Smrcok, B. Koppelhuber-Bitschnau, K. Shankland, W. I. F. David, D. Tunega and R. Resel (2001) *Z. Kristallogr.* ***216***, 63-66.

15.5 Tutorial 5 - Dealing with Two Molecules in the Asymmetric Unit

15.5.1 Introduction

The object of this tutorial is to guide you through the structure solution of uridine. It assumes that you have completed Tutorial 1. In this tutorial, you will learn:

- How to handle two molecules in the asymmetric unit
- To appreciate the importance of a good molecular model.



15.5.2 Data

The data set *Tutorial_5..xye* is a synchrotron X-ray diffraction data set collected on BM16 at the ESRF, at 130K. The incident wavelength was 0.85075 Å.

15.5.3 Stage 1: Reading the Data

- Open DASH and select the directory where the data resides.
- Select **View data / determine peak positions** and click **Next >**.
- Select the file *Tutorial_5.xye* using the **Browse...** button.
- Click **Next >**.
- Check that the wavelength and radiation source have been set correctly and click **Next >**.

15.5.4 Stage 2: Examining the Data

The data spans 4 to 30° 2q. Truncate the data to 2.0 Å resolution. The background can be removed

at this stage so proceed to do so. The default value for the window parameter of 100 is appropriate. When you are satisfied that the background fit (green line) is reasonable, click **Apply** and then **Next >**.

15.5.5 Stage 3. Fitting the Peaks to Determine the Exact Peak Positions

Select the first 23 peaks using the method described in Tutorial 1, Stage 3.

Here is a guide to the positions (° 2q) of the first 23 peaks:

4.84	6.63	7.07	7.51	7.81
9.70	10.51	10.56	10.77	10.91
11.12	11.40	11.98	12.11	12.21
12.52	12.77	13.22	13.28	13.74
14.10	14.30	14.42		

- Click **Next >**.
- Select **Run>** to run DICVOL or use another indexing program as described in Tutorial 1.

15.5.6 Stage 4. Indexing

If you have selected peaks which are very similar to those given in the previous stage the DICVOL program returns a monoclinic cell with a = 13.8703 Å, b = 14.7167 Å, c = 4.9207 Å, beta = 95.70° and cell volume = 999.47 $Å^3$ with figures of merit M(23) = 74.8 and F(23) = 374.8. Other cells are suggested with beta greater than 96° but it is customary to choose the cell with the smallest angle. (This turns out to be in very good agreement with a single crystal structure reported in the Cambridge Structural Database [CSD] reference code BEURID10.)

15.5.7 Stage 5. Stop and Think

Does the cell make sense? In this case we estimate the molecular volume to be 17 x 15 $Å^3$ (9C, 2N and 6O) + 12 x 5 $Å^3$ (12 H) = 315 $Å^3$. If there were 2 or 4 molecules in the unit cell we thus estimate volumes of 630 or 1260 $Å^3$ respectively. The estimate for 4 molecules per cell is more likely, allowing for the fact that there is likely to be extensive H-bonding which will tend to make the cell smaller in volume.

15.5.8 Stage 6. Checking the Cell and Determining the Space Group

You should check through the space groups (scrolling through the choices with the arrow keys) until a good match between the tick marks and peak positions is obtained. A very good correspondence is achieved with space group $P\ 1\ 2_1\ 1$, number 4:b. Thus we need to attempt structure solution in $P2_1$

with 2 independent molecules in the asymmetric unit.

15.5.9 Stage 7. Extracting Intensities

Pick 8 peaks which are isolated using the method described in Tutorial 1, Stage 7. When 8 peaks have been chosen the **Pawley Refinement Status** window will pop up automatically. The initial 3 cycles of the least squares refinement only involves the terms corresponding to the background. This should give a Pawley c^2 of 3 or better, accept these three cycles. The next 5 cycles of the least squares refinement should bring the Pawley c^2 down to about 1.5. Accept your best Pawley fit, making a note of c^2 and save the file as *Tutorial_5.sdi*.

15.5.10 Stage 8. Molecule Construction

Construct a 3D molecular description of the molecule using your favourite modelling software and save it in pdb, mol or mol2 format. Care must be taken with the conformation of the ribose ring. The five-membered ribose ring is not planar - four atoms of the ring define a plane and the 5th atom will be found either above the plane (on the same side as the 6-membered heteronuclear ring) or below the plane. If we search the CSD for molecules very similar to uridine, we find that the torsion angle defined by O, C4', C3' and C2' is either between 0 and +30° (C3' lies below the plane) or C3' lies above the plane (torsion angle between 0 and -40°. In uridine, C3' lies above the plane and to start with, your molecular model should copy this. Later, you will run through the simulated annealing process with a molecular model of a different ring conformation. If you do not have access to a molecular modelling package a *.mol2* file created by SPARTAN is included for the tutorial: *Tutorial_5.mol2*.

15.5.11 Stage 9. Setting up the Structure Solution Run

- Continue on from the Pawley fitting stage by selecting **Solve >**.
- Click on the  icon and select *Tutorial_5.mol2* (the file that you created in Stage 8); a Z-matrix file called *Tutorial__1.zmatrix* will be generated automatically.
- Read in the *Tutorial_5_1.zmatrix* file.
- As there are two molecules in the asymmetric unit, read in the *Tutorial_5.zmatrix* file again.

At this point DASH will confirm that there are 16 independent parameters. These parameters are listed when you click on **Next >**. There are 3 parameters describing the positional co-ordinates, 4 (of which 3 independent) describing the molecular orientation within the unit cell and 2 variable torsion angles, for each molecule.

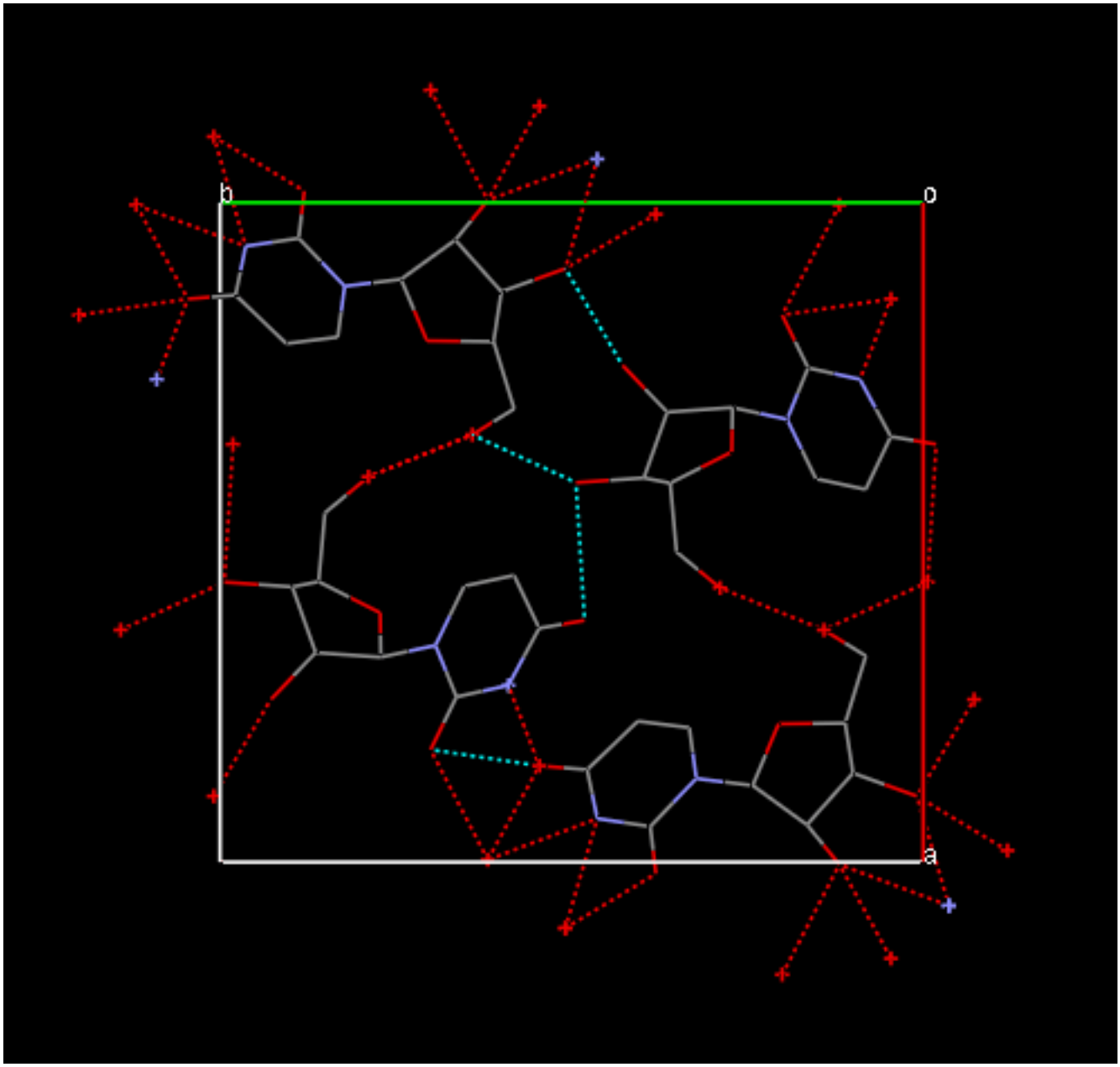
Click on **Next >**, leave the *Simulated Annealing Protocol* window with the default values, click **Next >** again and then click on **Solve >**. The structure should solve reasonably quickly.

15.5.12 Stage 10. Monitoring Structure Solution Progress

The progress of the structure solution can be followed by monitoring the profile c^2 and the difference plot. The molecule and crystal packing can be examined using the **View** button.

15.5.13 Stage 11. Examining the Output Structure

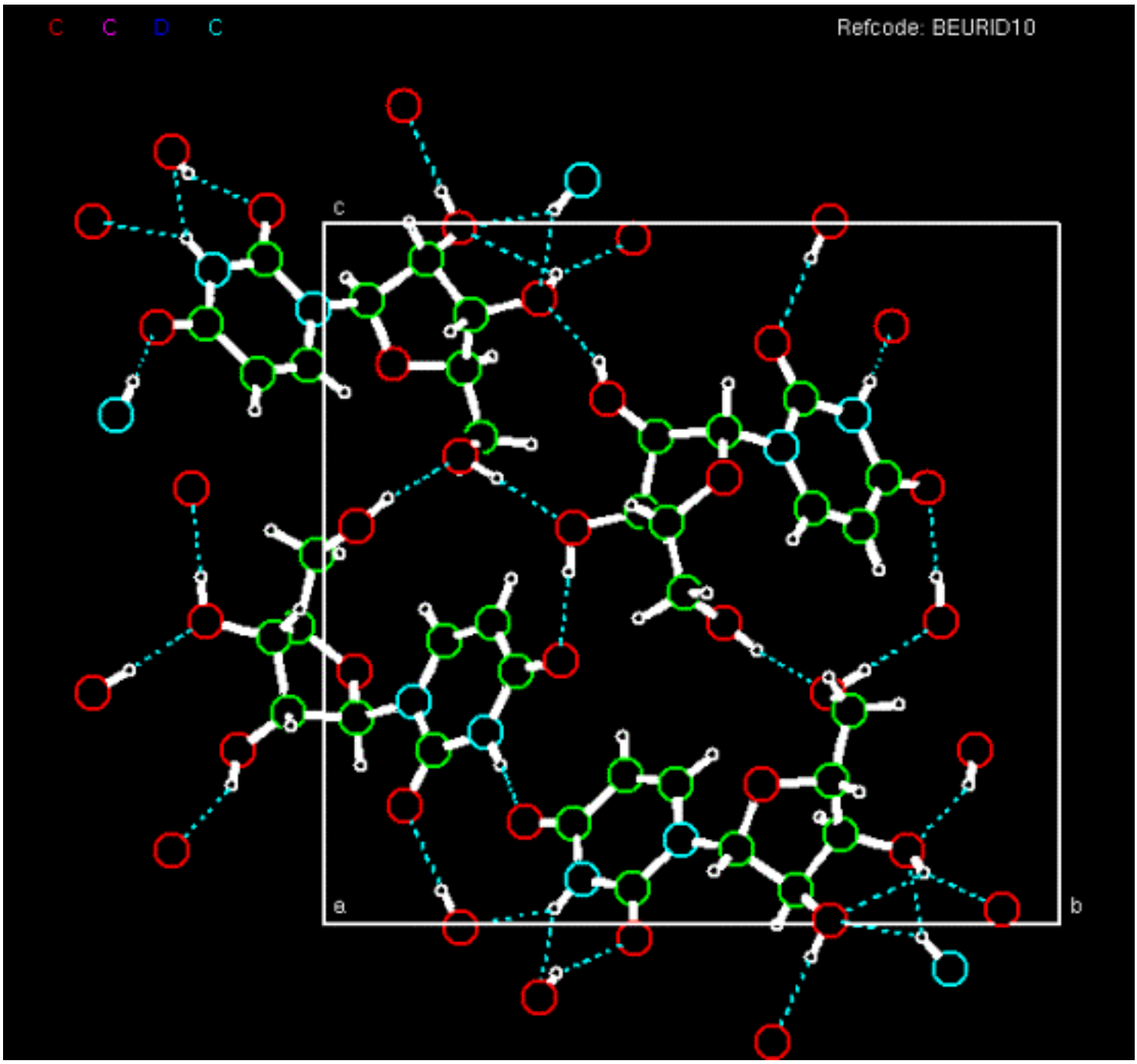
View the structure using the **View** button in the *Results from Simulated Annealing* window. All should look reasonable; there should be no abnormal close contacts between the atoms. In particular, check the formation of H-bonds. The picture below is taken from the Mercury visualiser for a SA good solution. In order to get a simple view of H-bonds we suggest clicking **Show hydrogens** off, and **H-Bond** on.



In the CSD it is found that in nearly every case H-bond donor atoms will be satisfied, so you should check all the OH groups, and the NH. Most of the O acceptor atoms will also take part in H-bonds, except for the ribose O which is often found not to accept. Note that in DASH the torsion angles involving H atoms are fixed by default at whatever value was input from the model - this means that the H-atoms do not necessarily point in the correct direction to form optimal H-bonds in these SA solutions.

For comparison an H-bond picture from Rpluto is given below for the single crystal structure BEURID10, which shows the same H-bond pattern. The directions of the axes may be inverted as absolute configuration cannot be determined from powder data. There is an ambiguity of 0.5 in the

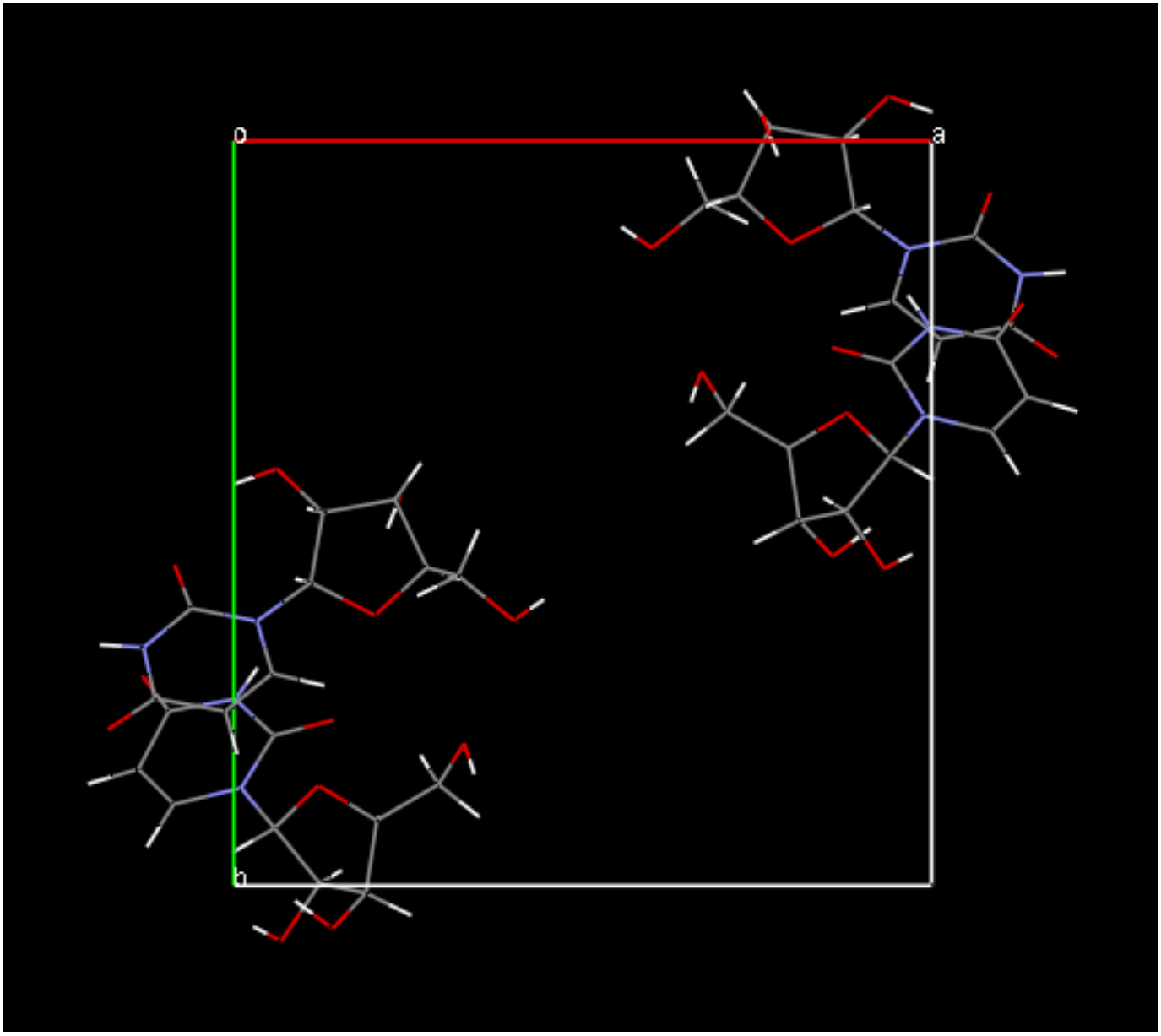
axial directions **a** and **c**, because of defined origin choice on a screw-axis, and the **b**-axis origin position is indeterminate.



15.5.14 Stage 12. Experiments with the Ring Conformation

When the molecular model was built, it was stressed that the ribose ring is puckered, with the 3' carbon out of the plane of the rest of the ribose ring and on the same side as the 6-membered ring. Build a model where the conformation of the ribose ring is different, for example where the 3' carbon of the ring points away from the 6 membered ring, the other four atoms of the ring defining a plane. If you do not have access to a model building package a file, *Tutorial_5-2.mol2* is included in the data

files. Repeat the Simulated Annealing stage (you can use DASH Wizard) but this time import *Tutorial_5-2.mol2* and then read in the newly created *Tutorial_5-2_1.zmatrix* file, as before. Proceed with the solution stage. You will find that a good solution is not found, and molecules may appear tangled, with close contacts. An example result is shown below:



It should also be mentioned that you can experiment in DASH with flexible ring systems only by a rather crude method, whereby one takes the molecular model in the model building program and breaks a bond in the ribose ring - say C2'-C3', and then export as a MOL2 file. Importing this into DASH then causes the rings to be treated as flexible chains, adding in fact 4 torsion angles per molecule; 8 torsion angles for the total SA search. This does not work in this case, as we have reached the present limit of the method with this data, but it is worth trying in less complex structures.

15.5.15 Stage 13. Conclusion

- DASH can solve structures with two molecules per asymmetric unit.
- The CSD should be consulted with regard to conformations of flexible rings.
- Models with markedly wrong ring conformations will not give the correct solution.

15.5.16 References

DICVOL Program:
D. Louer & M. Louer (1972) J. Appl. Crystallogr. ***5****, 271-275.*
A. Boultif & D. Louer (1991) J. Appl. Crystallogr. **24**, 987-993.

Model Builder:
PC Spartan Pro Version 1.0.5 (16/8/2000) Copyright (1996-2000) Wavefunction, Inc.

Visualiser:
Mercury (provided with DASH, has good H-bonding features)
RPluto (has H-bond graph set analysis features)
CCDC (1999). RPluto is freely downloadable for non-commercial purposes from *http://www.ccdc.cam.ac.uk/prods/rpluto/index.html*

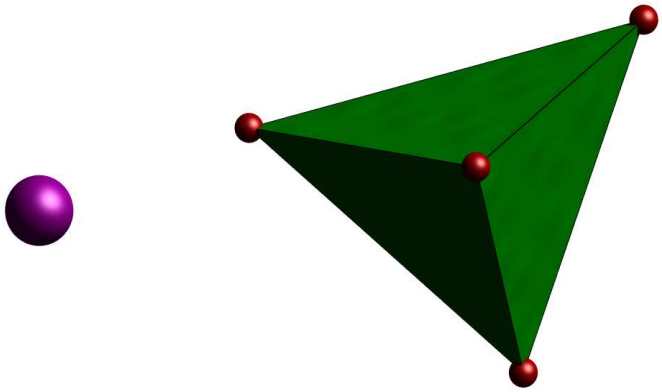
Single crystal structure (CSD reference code BEURID10):
E.A. Green, R.D. Rosenstein, R. Shiono, D.J. Abraham, B.L. Trus, R.E. Marsh (1975) *Acta Crystallogr.*, B**31**, 102-107.

15.6 Tutorial 6 - Solving the Structure of an Inorganic Compound

15.6.1 Introduction

The object of this tutorial is to guide you through the process of solving an inorganic crystal structure, using the compound ZrW_2O_8 as an example. This compound, zirconium bis(tungstate), contains zirconium ions (pink) and tungstate tetrahedra (green, with oxygen atoms shown in red). For this tutorial, it is assumed that you have already completed Tutorial 1. In the process of this tutorial you will learn how to:

- Solve the structure of an inorganic compound.
- Handle multiple structural fragments as separate z-matrices.
- Cope with the complications of high symmetry space groups.



15.6.2 Data

The data set *Tutorial_6.raw* is a laboratory x-ray diffraction data set collected at room temperature by John Evans. The incident wavelength was 1.54060 Å.

15.6.3 Stage 1: Reading the data

- Open DASH and select the directory where the data resides.
- Select View data / determine peak postions and click Next >.
- Select the file *Tutorial_6.raw* using the Browse... button.
- Click **Next >**.
- Check that the wavelength and radiation source have been set correctly and click **Next >**.
- The default settings shown in the *Background Subtraction* window are good enough for this simple background. Click **Next >**.

15.6.4 Stage 2: Examining the Data

This data set is very clean, with a very low background and sharp reflections, so we do not want to throw away any of the high resolution reflections. Change the settings to truncate the data to a resolution of 1.0 Å. Subtract the background using the default window setting of 100. Click **Next >**.

15.6.5 Stage 3. Fitting the Peaks to Determine the Exact Peak Positions

Select the first 20 peaks using the method described in the first tutorial.

Here is a guide to the approximate positions (2q) of the first 20 peaks:

16.7803	19.3916	21.6881	23.7869	27.5389
29.2538	30.8623	32.4090	35.3199	36.7033

39.3372	40.5986	41.8329	43.0303	44.2036
45.3575	46.4954	48.6816	49.7570	50.8086

15.6.6 Stage 4. Indexing

A typical run of DICVOL, if the selected peaks were very close to those given in the previous stage, should return a cubic cell as the best fit, with:

a = b = c = 9.1547 Å, V = 767.23 $Å^3$

Figures of merit: M(24) = 160.1, F(24) = 154.6

A number of other possible cells are likely to appear with lower symmetry. Select the cubic cell as this should give the best figures of merit.

15.6.7 Stage 5. Stop and Think

Does the cell make sense? There is a very approximate method of estimating molecular volume using 15 $Å^3$ per C, N, O atom and 25 $Å^3$ for heavier atoms. So for this compound, ZrW_2O_8, we estimate the formula unit volume to be 195 $Å^3$, so 4 formula units per cell would need a volume of approximately 780 $Å^3$. The DICVOL cell volume of 767 $Å^3$ suggests that we have roughly four formula units per cell.

15.6.8 Stage 6. Checking the Cell and Determining the Space Group

The space group that is automatically selected for the cubic crystal system is *P*23. A quick scan of the diffraction pattern and tick-marks for the predicted reflections should show that the majority of tick-marks correspond to observed peaks. This suggests that the space group is either correct, or quite close to the correct one. Try selecting some of the other space groups in the list for the cubic crystal system and looking at the correlation between tick-marks and peaks. You will see that the majority of space groups produce regions where there are no tick-marks, but there are observed peaks, e.g. *F*23, *I*23, $I2_13$, *Fm*-3, *Pa*-3.

The correct space group for this crystal structure is actually $P2_13$. Have a look at the predicted peak positions for this space group and check that these correspond to the observed peaks. To check that this is correct, run the space group determination tool. This should confirm that the space group is primitive and that the only systematic absences present indicate a 2_1 screw axis, which only leaves two possible space groups - $P2_13$ and $P4_232$. The structure will not solve, however, in $P4_232$.

15.6.9 Stage 7. Extracting Intensities

Select a series of isolated peaks across the diffraction pattern (e.g. 16.77, 21.69, 32.41, 45.36, 51.85, 62.50) as shown in previous tutorials. The peak picking algorithm may continue to the Pawley refinement step after you have only chosen 5 or 6 peaks if it deems the peaks chosen acceptable. The initial 3 cycles of refinement should give a Pawley c^2 of around 1.2; accept these three cycles. The next five cycles of least squares refinement should bring the Pawley c^2 value down slightly further. Refinement of the peak shape parameters is unlikely to improve the refinement for this data set. Your final c^2 parameter should be in the region of 1.0 - 1.2.

Accept your best Pawley fit, making a note of the c^2 value, and save it as *Tutorial_6.sdi*.

15.6.10 Stage 8. Building the Preliminary Model

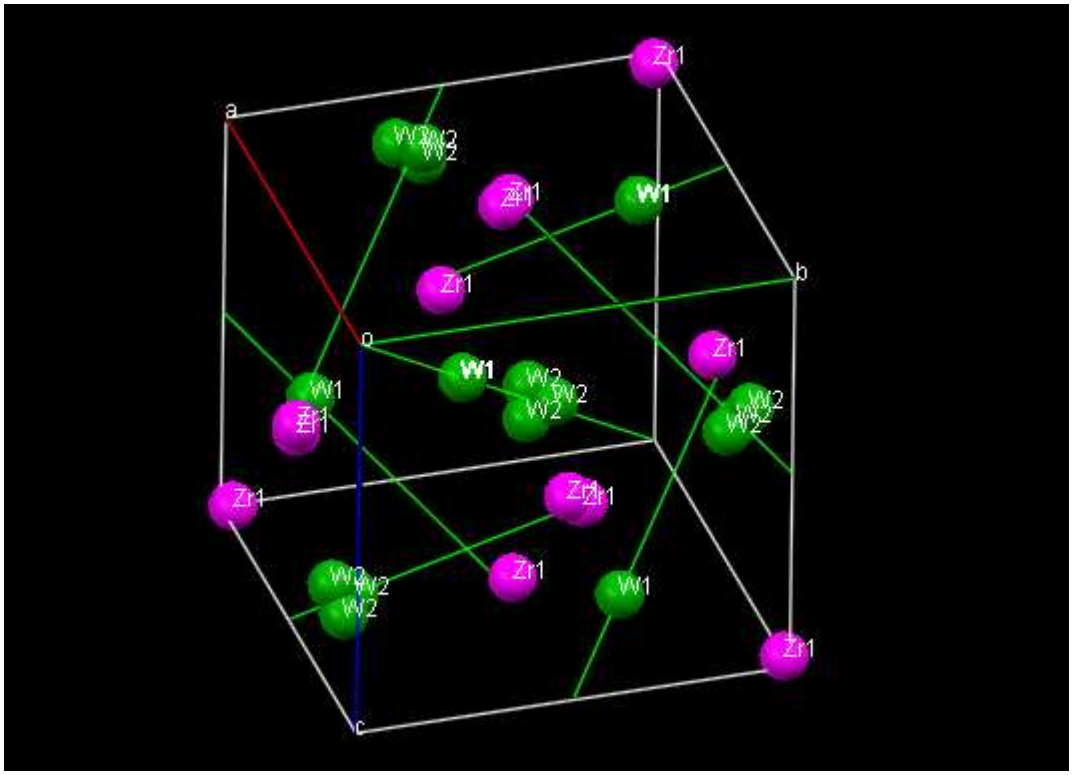
In order to solve the crystal structure we need to define a starting model. The oxygen atoms have much less electrons than the Zr and W atoms and therefore scatter x-rays more weakly. This means that it is sensible to determine the positions of the heavy atoms first before attempting to determine the light atom positions. A starting model for the heavy atom structure solution can therefore be defined as simply free Zr and W atoms. These can be set up using your preferred model building software, or using a file supplied with the tutorial, *Tutorial_6-atoms.mol2*.

15.6.11 Stage 9. Setting up the Structure Solution Run

- Start DASH as before and select Simulated annealing structure solution from the Wizard.
- Select the *Tutorial_6.sdi* file.
- Click on the  icon and select *Tutorial_6-atoms.mol2* (the file that you created in Stage 8); three Z-matrix files will be automatically generated.
- At this point DASH will identify that there are 9 independent parameters; three parameters each for the positional coordinates of the three atoms (1 Zr atom and 2 W atoms). The parameters will be then be listed along with their initial values and parameter bounds when you click on **Next >**. Note that each atom is free to move anywhere in the unit cell and that each atom has a full occupancy. As DASH does not have 'anti-bumping' constraints, multiple atoms are free to move to the same site.
- Click **Next > to access the Simulated Annealing Protocol window**. Change the Profile chi-sqd multiplier to 6.0, leave the remaining variables at their default values, click **Next >** and then click **Solve >**. This set of SA runs will take a little while, but should converge eventually to a solution with a profile c^2 in the region of 5.75. Ideally each SA run should produce a solution with approximately the same value of c^2, suggesting that this is the correct solution and is reproducible.

15.6.12 Stage 10. Analysis of Preliminary Solution

- Take a look at the solutions found by DASH individually by clicking on the View buttons for each row in the Analyse solutions table.
- It should be obvious that the Zr atoms are aggregating on the origin, or one of the symmetry equivalent positions to the origin in each case (e.g. 0, 0.5,0 or 0.5, 0, 0.5 etc.).
- The W atoms are also seen to aggregate, but these sit on the 3-fold axes of the unit cell. This can be seen by turning on the symmetry elements in Mercury - click Display > Symmetry Elements... The 3-fold axes should be shown as green vectors with no arrows on.
- In this case the fact that the atoms were each assigned full occupancy, rather than the correct occupancy (which should be 1/3 as they each reside on the 3-fold axis) was not a problem because the ratios of the atomic occupancies are correct. The incorrectly high occupancies used are compensated by changes in the scale factor. Once the oxygens are included it will become important to use the correct occupancies of 1/3 for the Zr and W atoms.



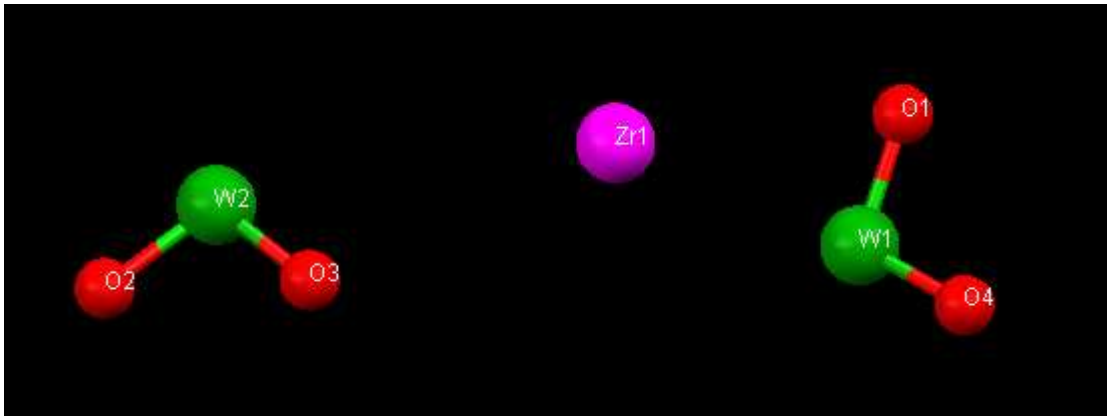
- The fit to the experimental diffraction pattern still shows a lot of differences as we have not found the positions of the oxygen atoms.
- We can now use these positions that we have determined for the heavy atoms in the next cycle of the structure solution in order to find the light atom positions.
- The coordinates of a general position on the 3-fold axis can be written as (x, x, x) - we have seen that the Zr atom resides at the position (0, 0, 0). The positions of the two W atoms can be found by clicking in Mercury on More Info > Atom List... Inspection of the W coordinates should

show that the average positions of the two atoms are (0.6, 0.6, 0.6) and (0.3412, 0.3421, 0.3412) respectively.

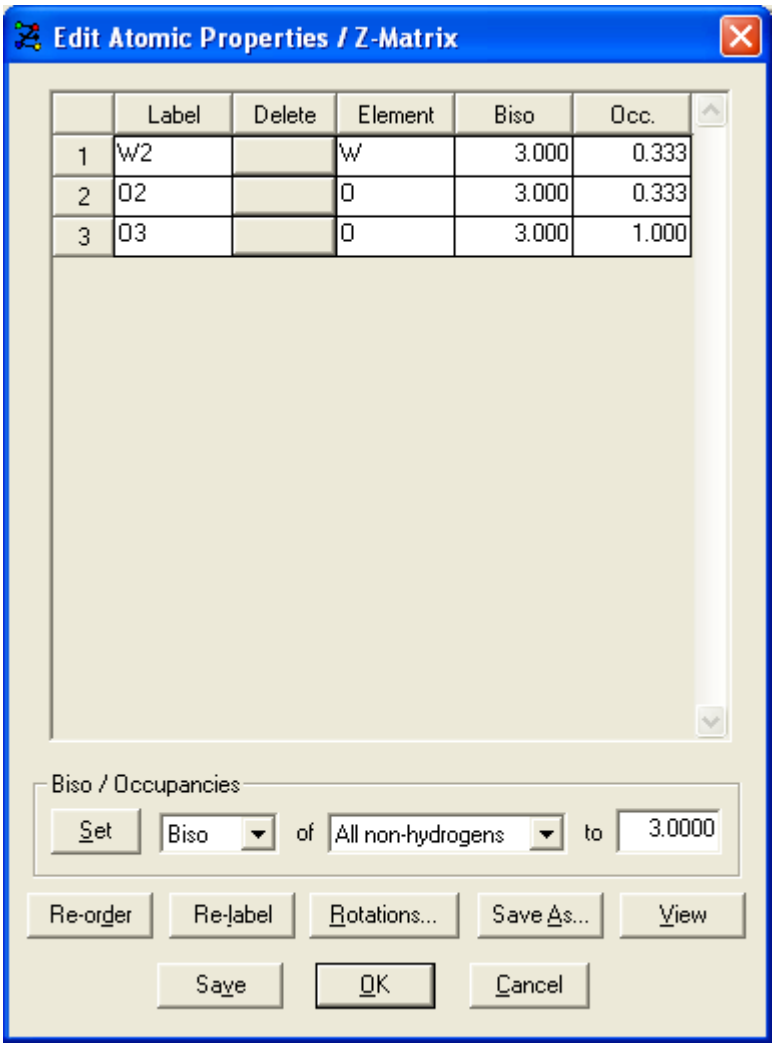
- Click < Back to return to the DASH Wizard welcome window and select Simulated annealing structure solution. Click Next > to continue.

15.6.13 Stage 11. Building the Full Model

- In order to fully solve the structure and determine the light atom positions we now need to set up a more complicated structural model. We will continue to use a free Zr atom and use a tetrahedral geometry for the tungstate moieties. As the W atoms are each on a 3-fold axis, the rest of the tungstate tetrahedra must be represented by one partially occupied O atom on the 3-fold axis and one fully occupied O atom lying off the 3-fold, with the remaining two O atoms generated by symmetry.
- For the tungstate tetrahedra we can therefore use O-W-O fragments generated with ideal O-W bond lengths and an ideal tetrahedral O-W-O bond angle. These fragments can be generated using your preferred model building software, or using a file supplied with the tutorial, *Tutorial_6-atoms.mol2*.



- The structure solution window will still show the same `.sdi` file as used previously along with the atom z-matrices which we used in the preliminary solution.
- Click on the  icons to remove each of the atomic z-matrices. Next, click on the  icon and select *Tutorial_6-frags.mol2*; three Z-matrix files will be automatically generated.
- We also need to edit the occupancies of the atoms on special positions. To do this, click Edit in the row for the z-matrix. The atoms Zr1, W1, O1, W2 and O2 should be set as an occupancy of 0.3333 and the remaining two atoms (O3 and O4) should be left with a occupancy of 1.0 (e.g. see below).

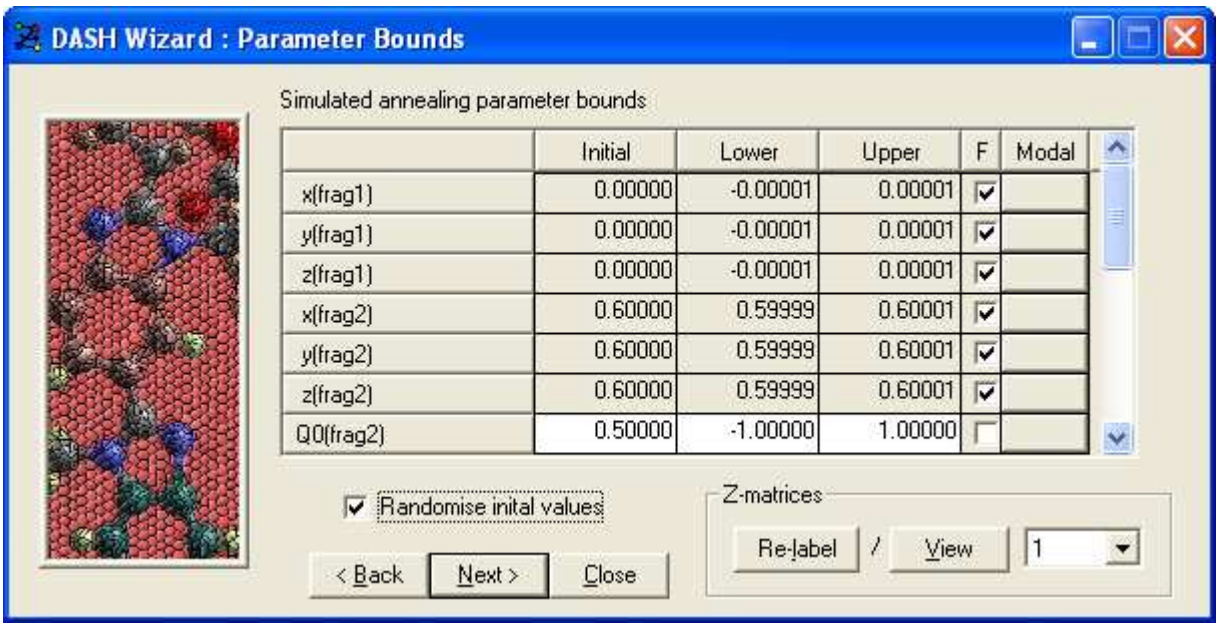


- The DASH Wizard will also confirm that you now have 15 independent degrees of freedom. Click **Next >** to continue.

15.6.14 Stage 12. Setting up the Full Model Structure Solution

- In the Parameter Bounds window we now want to use the information gained during the preliminary solution to fix the coordinates of the heavy atom positions.
- The Zr atom, as found earlier, sits on the origin. Fragment 1 corresponds to the free Zr atom, so we can change the initial x(frag1), y(frag1) and z(frag1) values to 0.0 and click on the checkbox in the F column for each of these rows to fix the coordinates.
- Fragments 2 and 3 correspond to the two O-W-O fragments and we can fix the coordinates of the W atoms by simply putting in the initial values of x, y and z for fragments 2 and 3 as the W atom corresponds to the origin coordinates for the fragment.
- Choose the coordinates of fragment 2 to be (0.6, 0.6, 0.6), fragment 3 to be (0.3412, 0.3412, 0.3412) and set these parameters to be fixed as well using the checkboxes in the F column. The

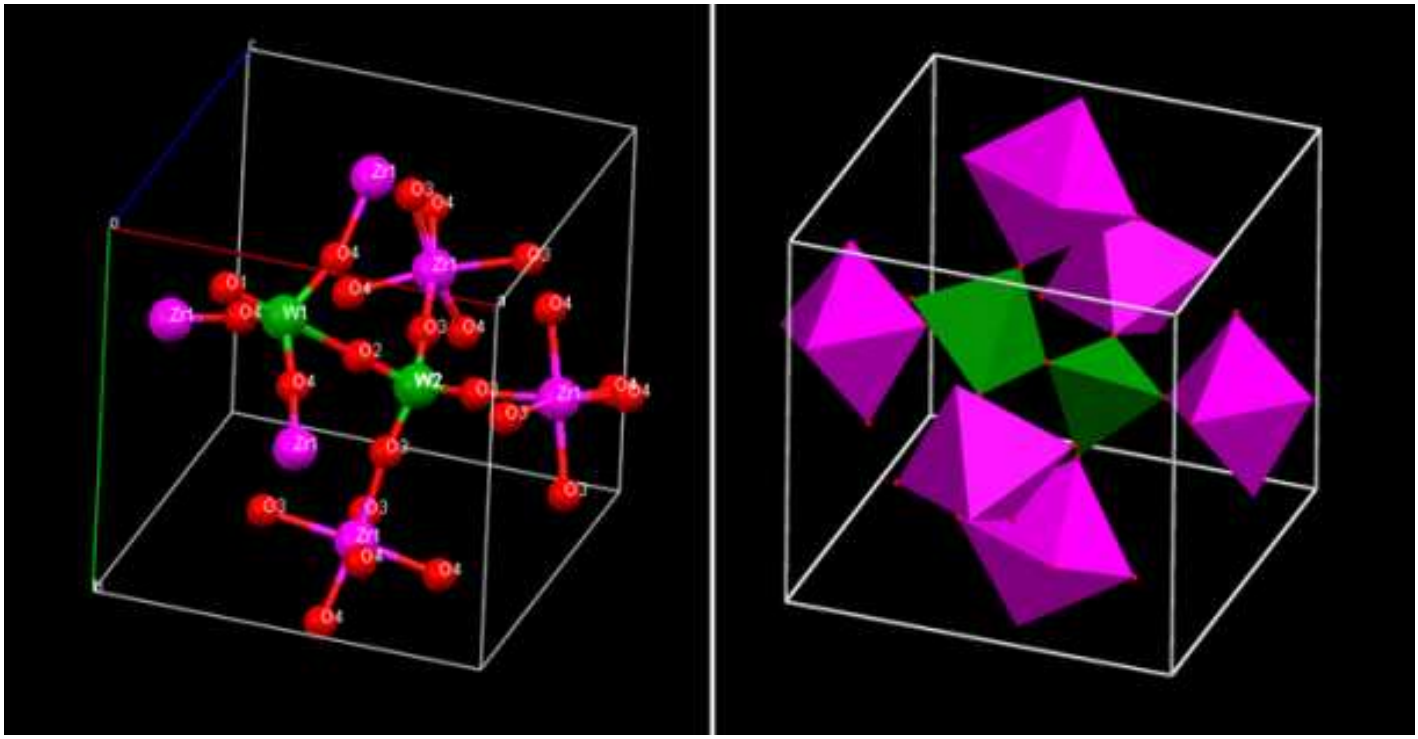
rows for these fixed parameters should now all be greyed out (see figure below).



- Click Next > to continue.
- In the Simulated Annealing Protocol window, choose a lower Profile chi-sqd multiplier (for example 1.5 or 2.0) this time as we expect the fit to the experimental data to be even better now as we are modelling the full structure. Also the O atoms have so few electrons compared to the Zr and W atoms, which means that the O atom positions only have a small effect on the diffraction pattern, so the correct solution is harder to find.
- Click **Next >** and then **Solve >** to start the Simulated Annealing process.
- This SA run is also likely to take a while as the differences between correct and incorrect solutions are quite small in terms of the effect on the profile.

15.6.15 Stage 13. Examining the Final Structure

- The Analyse solutions window will now show the results of the SA runs. The runs may not all have reached the same structural minimum and this can be seen by the range of final Profile chi-sqd values for the runs. Correct solutions should be identified by the lowest Profile chi-sqd and Intensity chi-sqd values.
- Take a look at the solutions found by DASH for the full structure by clicking on the View button for one of the top few solutions. Using the Packing feature of Mercury turned on, it should be possible to see that the tungstate moieties have formed into nicely shaped tetrahedra, although the O1 and O2 atoms may be slightly off the 3-fold axes. The ZrO_6 moieties are also formed into undistorted octahedra.
- The following figure shows an image of the solved structure in Mercury and the equivalent view displayed using ZrO_6 and WO_4 polyhedra in the DIAMOND structural visualiser.



- Clicking on the Show overlay button in the Analyse Solutions window should also show that the solutions fit with each other very well, which suggests that the solution is reproducible.
- In order to refine the structure in a meaningful manner there is a built-in rigid-body Rietveld refinement module (see Section 11.1 of the DASH User Guide). To start a Rietveld refinement from one of the structure solutions simply click on the Rietveld button for that row of the Analyse Solutions table and choose the refinement package that you wish to use.
- Careful refinement of the bond angles and bond distances should cause the tungstate moieties to assume a better shape.

15.6.16 References

DICVOL Program:
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A. Boultif & D. Louer (1991) J. Appl. Crystallogr. ***24****, 987-993.*

Extinction Symbol Program:
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Model Builder:
WebLabViewerLite Version 3.20 (12/8/98) Copyright 1998 Molecular Simulations, Inc.

Powder crystal structure ZrW_2O_8 *(ICSD code 83267):*

*T. A. Mary, J. S. O. Evans, T. Vogt & A. W. Sleight (1996) Science **272**, 90-92.*

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